Asymmetric synthesis of 2-deoxystreptamine analogues as potential RNA ligands

Guuske F. Busscher

Asymmetric synthesis of 2-deoxystreptamine analogues as potential RNA ligands

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door

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Nothing great was ever achieved without enthusiasm
Ralph Waldo Emerson (1803-1882)

Voor Opa Prak

Paranimfen Jantine F. Busscher
Rosalie L. M. Teeuwen

List of Abbreviations

Ac	acetyl
ACD	available chemical directory
Ac_2O	acetic anhydride
AcOH	acetic acid
AD	asymmetric dihydroxylation
AIBN	2,2'-azobisisobutyronitrile
Ala	alanine (= A)
All	allyl
Arg	arginine (= R)
Asn	asparagine (= N)
aq	aqueous
AAC	aminoglycoside acetyltransferases
AAD	aminoglycoside adenylyltransferases
ANT	aminoglycoside adenylyltransferases
APH	aminoglycoside phosphotransferases
Bn	benzyl
Boc	*tert*-butoxycarbonyl
BORSM	based on recovered starting material
br	broad (NMR)
BSP	1-benzenesulfinyl piperidine
*btr*C	gene in *bacillus circulans*
Bz	benzoyl
ºC	degrees Celcius (centigrade)
CAN	ceric ammoniumnitrate
CDI	carbonyldiimidazole
CI	chemical ionization
CSA	camphorsulfonic acid
Cbz	benzyloxycarbonyl
Compd.	compound
d	doublet (NMR)
d	days
DACH(s)	diazidocyclohexitol(s)
dba	dibenzylidene acetone
DBU	1,8-diazabicyclo [5.4.0]undec-7-ene
d.e.	diastereomeric excess
DCC	*N*,*N*-dicyclohexylcarbodiimide
DDQ	2,3-dicyano-5,6-dichloro-parabenzoquinone
DIBALH	di*iso*-butylaluminium hydride
Dipea	di*iso*propylethylamine
DMAP	4-(dimethylamino) pyridine
DMF	*N*,*N*-dimethylformamide
DMP	2,2-dimethoxypropane
DMSO	dimethyl sulfoxide
DOI	2-deoxy-D-*scyllo*-inosose
DOIS	*2-deoxy*-scyllo-*inosose synthase*
2-DOS	2-deoxystreptamine
dppf	diphenylphosphino ferrocene
DPPB	1,4-(diphenylphosphino) butane
EDT	ethanedithiol
e.e.	enantiomeric excess
e.g.	*exempli gratia* (for the sake of example)
EI	electron impact
e.p.	enantiopure
ESI	electrospray
et al.	*et aliae* (and others)
EtOAc	ethyl acetate
Et_2O	diethyl ether
equilibr.	equilibrium
equiv	equivalents
FVT	flash vacuum thermolysis
Fmoc	9-fluorenyl methoxycarbonyl
h	hours
HIV	human immunodeficiency virus
His	histidine (= H)
HOBt	*N*-hydroxybenzotriazole
HPLC	high performance liquid chromatography
i.e.	*id est* (that is)
LTT	2-lithiothiazole
MALDI-TOF	matrix-assisted laser desorption ionization-time of flight mass spectrometry
m-CPBA	*meta*-chloroperoxybenzoic acid
Me	methyl
MeCN	acetonitrile

min	minutes
mRNA	messenger ribonucleic acid
MS	mass spectroscopy
N	normal (equivalents per liter)
NAD	nicotinamide adenine dinucleotide
NADH	reduced NAD
NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance
o	ortho
OSu (ester)	hydroxysuccinimide
p	para
Ph	phenyl
Phe	phenylalanine (= F)
Piv	pivaloyl
Pmc	2,2,5,7,8-pentamethyl-chromane-6-sulfonyl
ppm	parts per million
PyBoP	(benzotriazol-1-yloxy) tripyrrolidinophosphonium hexafluorophosphate
PyBroP	bromotris(pyrolidinone) phosphonium hexafluorophosphate
q	quartet (NMR)
r	ribosomal
Ra-Ni	Raney nickel
RCM	ring closing metathesis
RNA	ribonucleic acid
RRE	Rev response element
rt	room temperature
s	singlet (NMR)
Ser	serine (= S)
t	triplet (NMR)
TBDMS	*tert*-butyldimethylsilyl
t-Bu	*tert*-butyl
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
Tf_2O	trifluoromethanesulfonic anhydride
THF	tetrahydrofuran
TIS	tri*iso*propylsilane
TMS	trimethylsilyl
Trt	trityl
Ts	*p*-toluenesulfonyl
TTBP	2,4,6-tri-*tert*-butylpyrimidine
Tyr	tyrosine (= Y)
U-MCR	Ugi multi component condensation reaction
Val	valine (= V)
VCD	vibrational vircular dichroism
viz.	*videlicet* (it is permitted to see)

Contents

Preface

Undoubtedly, the most important discovery in the field of medicine is the discovery of penicillin by Sir Alexander Fleming (1928). Prior to that time, bacteria were responsible for many of the world's lethal diseases such as pneumonia, plague and tuberculosis, together responsible for killing more people than any other disease. With the advent of antibiotics –many others rapidly followed after penicillin– few people in the Western world nowadays consider bacterial infections as life–threatening as for instance viral infections, heart diseases or cancer.

However, the ever-increasing resistance of bacteria against commonly used antibiotics – along with a growing number of infectious diseases– becomes a situation of most concern (Figure 1). Also in Europe, most of the microbes responsible for causing invasive infections have already reached alarmingly high resistance percentages, *e.g.* 26% for methicillin resistant *S. aureus* (MRSA), 12% for erythromycin non-susceptible *S. pneumoniae* (ENSP) and 9% for vancomycin resistant enterococci (VRE). The most worrying trend in Europe is noted for *E. coli* (Table 1), the most common cause of infections due to Gram-negative bacilli and the principal pathogen in urinary tract infections. At the same time, there is a continuous decline of interest of the pharmaceutical industry in the development of novel antibiotics.[1] As a result, no truly new antibiotics have emerged since the discovery of the fluoroquinolones in the early 1970s, with the single exception of Zyvox™ (linezolid), the first of the class of oxazolidinones, approved for treatment of Gram-positive infections by the FDA in 2000.[2,3]

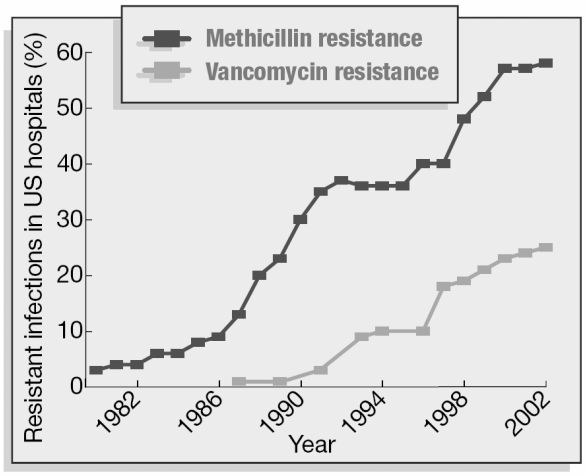

Figure 1. Historic development of resistance against methicillin and vancomycin in U.S. hospitals.[4]

antibiotic	% resistance	% increase (from 2001)
aminopenicillins	51	19
aminoglycosides	6	36
fluoroquinolones	16	82
3rd gen. cephalosporins	3	113

Table 1. Resistance of *E. coli* in Europe (2005) against commonly applied antibiotics and comparison with 2001.[5]

Another class of antibiotics with a broad antibacterial spectrum and proven efficacy particularly against aerobic Gram-negative bacteria is formed by the aminoglycosides. Although resistance of some bacteria has reached dramatic levels, *e.g.* an average of 66% for *E. faecalis* in Europe, with several enterococci phenotypes approaching 100% in some countries (Italy, Romania, Germania). The prime resistance mechanisms involve structural modification by bacterial enzymes; aminoglycoside phosphotransferases (APH), adenyltransferases (AAD or ANT) and acetyltransferases (AAC).[6,7] Apart from that, extensive clinical use of the aminoglycosides is limited due to the associated nephro- and ototoxicities.[8] These circumstances validate research into novel aminoglycoside analogues which do not display the undesirable features but maintain a strong bactericidal effect by evading resistance mechanisms. This notion has already awakened the chemical community and the number of papers along this line is rapidly expanding. Omnipresence in the aminoglycoside family is a cyclohexitol moiety termed 2-deoxystreptamine (2-DOS). An efficient route to obtain 2-DOS may therefore serve as a practical starting point for the assembly of aminoglycoside-type libraries. An overview of the synthetic routes leading toward 2-DOS that appeared in literature earlier is given in chapter 1.

Unfortunately, all synthetic routes toward 2-DOS that have been developed thus far require expensive starting material or numerous synthetic steps and offer minimal flexibility in protective groups. As a result, to date the most practical method to synthesize 2-DOS (derivatives) is *via* degradation of natural neomycin. However, the initially obtained "naked" *meso*-compound still demands desymmetrization as well as extensive protective group manipulations before incorporation in aminoglycoside entities can be ensured. The aim of the research described in this thesis is to first develop a fast route toward 2-DOS precursors (chapter 2-5) and secondly to synthesize from such a core structure a variety of aminoglycoside analogues (chapter 5 and 6). In the ideal scenario, a 2-DOS precursor was projected suitable to serve as scaffold for both 4,5- and 4,6-linked aminoglycoside antibiotics. Consequently, our research initially focused on the synthesis of orthogonally protected derivatives of 2-DOS, starting from enantiomerically pure D-allylglycine (chapter 2). The following chapter 3 describes an alternative synthetic approach toward 2-DOS commencing with a Diels–Alder condensation of cyclopentadiene and *p*-benzoquinone. The most straightforward strategy leading to *meso* 5-*O*-benzylprotected diazidocyclitol precursors of 2-DOS employs commercially available kanamycin B sulfate as starting material (chapter 4). In the same chapter, several methodologies are explored to obtain enantiopure 1,3-diazidocyclohexitols (1,3-DACHs) *via* resolution of chiral intermediates or the final *meso* 5-*O*-protected precursor, for example by asymmetric alkylation, asymmetric allylic alkylation or enzymatic hydrolysis.

The synthesis of a carbohydrate mimic of 2-DOS starting from D-ribose is described in chapter 5. Moreover, glycosylation of the carbohydrate 2-DOS derivative with phenyl thioglycoside in the presence of TTBP and AgOTf followed by ring-closing metathesis yielded a conformationally restricted aminoglycoside analogue. In the final chapter 6 the synthesis of 4,6-linked carbohydrate and peptidyl 2-DOS derivatives from *meso* 2-DOS precursors is described as a potential new class of RNA ligands.

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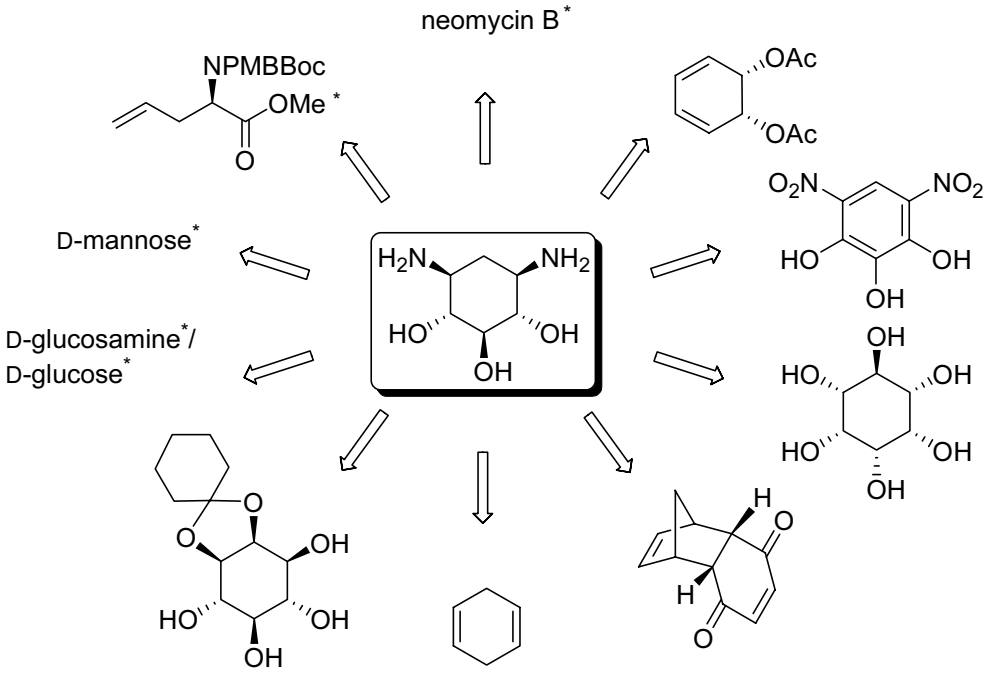
1 2-Deoxystreptamine, central scaffold of aminoglycoside antibiotics[1]

Abstract

2-Deoxystreptamine (2-DOS) is a common structure in nearly all of the aminoglycosides, a class of clinically important antibiotics, but with limited practical value due to high associated toxicities as well as microbial resistance. A possible strategy to develop new aminoglycoside antibiotics that do not display these undesirable features but maintain a strong bacterial effect starts from individually prepared components, such as 2-DOS. Therefore, an overview of synthetic routes leading toward this aminocyclitol moiety will be described in this chapter.



[1] Part of this chapter has been published in: G. F. Busscher, F. P. J. T. Rutjes, F. L. van Delft, *Chem. Rev.* **2005**, *105*, 775-791.

1.1 Introduction

Since the discovery of the aminoglycoside antibiotic streptomycin in 1944,[1] the family of aminoglycosides has steadily grown into a powerful class of antibiotics. Aminoglycosides form a large class of clinically important antibiotics with a broad antibacterial spectrum and proven efficacy, particularly against aerobic Gram-negative bacteria. (Table 1).[2] Most of the aminoglycosides are naturally occurring substances and are readily obtained from actinomycetes of either genus *Streptomyces* (labeled "-mycin") or *Micromonospora* (labeled "-micin").[3] The most relevant members of the family are streptomycin, tobramycin and gentamicin, the latter of which is used in the clinic most frequently due to its low cost and reliable activity. In general, aminoglycosides show a strong synergistic effect upon co-administration with penicillin, a particularly useful combination for treatment of patients with infections of unknown origin, although recent scientific reports appear to contradict the added value of co-administration.[4] Some semi-synthetic derivatives, *i.e.* amikacin, netilmicin, arbekacin, isepamicin, and dibekacin, are also on the market and display particular activity against bacterial strains that have developed resistance against the early aminoglycosides.[5] However, in recent years it has become apparent, after a long era of marginal interest in the search and development of antibacterials, that the battle against pathogenic bacteria is not over. In contrast, resistant strains of bacteria are reported with increasing frequency, with the associated dramatic consequences like untreatable patients or the temporary closure of hospital intensive cares.[6]

Apart from being versatile antibiotics, recent years have seen a shift of scientific interest in aminoglycosides to a related field of interest that has the potential of much broader application: the regulation of protein production at the RNA-level. Due to the fact that aminoglycosides are rather promiscuous ligands for all sorts of RNA, often in low micromolar concentration, they have earned themselves a strong position in the emerging research field 'RNA as a drug target': the perception that RNA plays a central (and active) role in the biochemical pathway required to produce proteins from DNA provides nearly untapped opportunities for biological and pharmacological development. For example, RNA is the genetic material of pathogenic viruses such as HIV or hepatitis C. Apart from that, the complex functions of RNA molecules in the control of gene expression in humans provide numerous opportunities to target specific RNA structures for treating a variety of chronic and degenerative conditions.[7,8]

Table 1. Aminoglycosides in clinical use

aminoglycoside	year of intro-duction	effective against bacterial strain[a]	disease(s) associated with bacteria
streptomycin	1944	*M. tuberculosis*	tuberculosis
		E. histolytica	diarrhea
		C. parvum	diarrhea
		F. tularensis	tularemia
		Y. pestis	plague
neomycin	1949	*Enterobacteriaceae spp.*[c] (including E. *coli*, *Klebsiella* spp[c]. etc)	several infections, sepsis
		Salmonella spp.[c]	diarrhea
		S. aureus	wound infections, sepsis, toxic shock syndrome
kanamycin	1957	Enterobacteriaceae spp.[c]	several infections, sepsis
		P. aeruginosa[b]	outer ear infection, sepsis, long infections
paromomycin	1959	*E. histolytica*	diarrhea
		C. parvum	diarrhea
spectinomycin	1962	*N. gonorrhoeae*	gonorrhea
		P. aeruginosa[b]	outer ear infection, sepsis, long infections
gentamicin	1963	*P. aeruginosa*[b]	outer ear infection, sepsis, long infections
		Enterobacteriaceae spp.[c]	several infections, sepsis
tobramycin (nebramycin factor 6)	1967	Enterobacteriaceae spp.[c]	several infections, sepsis
		P. aeruginosa[b]	outer ear infection, sepsis, long infections
sisomicin[d]	1970	Enterobacteriaceae spp.[c]	several infections, sepsis
		P. aeruginosa[b]	outer ear infection, sepsis, long infections
		S. *aureus*	wound infections, sepsis, toxic shock syndrome
dibekacin	1971		
amikacin[d]	1972	Enterobacteriaceae spp.[c]	several infections, sepsis
		P. aeruginosa[b]	outer ear infection, sepsis, long infections
		M. tuberculosis	tuberculosis
		MOTT	opportunistic infections
		N. asteroides	opportunistic infections
netilmicin[d]	1975	Enterobacteriaceae spp.[c]	intestinal infections
		P. aeruginosa[b]	outer ear infection, sepsis, long infections
isepamicin[d]	1978		nosocomial pneumonia
arbekacin[d]	1990		

[a] *M. tuberculosis=Mycobacterium tuberculosis, E. histolytica=Entamoeba histolytica, C. parvum=Cryptosporidium parvum, F. tularensis=Francisella tularensis, Y. pestis=Yersinia pestis, E. coli=Escherichia coli, S. aureus=Staphylococcus aureus, P. aeruginosa=Pseudomonas aeruginosa, N. gonorrhoeae=Neisseria gonorrhoeae, MOTT=mycobacterium other than tuberculosis, N. asteroids=Nocardia asteroids.* [b] Tobramycin and sisomicin show a better activity against *Pseudomonas aeruginosa* [c] spp.= species [d] semi-synthetic adducts.

Mechanism of action

Aminoglycoside antibiotics bind to the A-site decoding region of the bacterial 16S ribosomal RNA.[9,10] Since the natural role of the ribosome is to provide an environment for protein production, the effect of binding is expressed in mistranslation of mRNA or premature termination of protein synthesis, leading to cell death. In a more general sense, the aminoglycosides are found to have a broad, rather promiscuous preference for binding to A-form nucleic acids.[11] An interesting observation of this phenomenon is the binding of neomycin with HIV RRE, as a competitive inhibitor of the natural protein ligand Rev, resulting in attenuated HIV replication in tissue culture cells.[12,13,14]

Toxicity

Despite the apparent advantages, extensive clinical use of the aminoglycoside antibiotics is limited due to the associated toxicities, most notably nephrotoxicity and ototoxicity and to a lesser extent neuromuscular blockade.[3] The exact mechanism of toxicity is unknown although aminoglycosides are known to accumulate in renal cortical cells and are able to damage proximal tubules. Nephrotoxicity is dose-dependent and generally reversible in the majority of patients when the drug is discontinued. Of higher impact is the associated ototoxicity that may lead, depending on the phenotype of a particular patient, to irreversible vestibular and/or cochlear damage.

Resistance

Another and arguably more alarming drawback of the aminoglycosides (and antibiotics in general), is the global development of microbial resistance. In case of the aminoglycosides, which are used predominantly against aerobic Gram-negative bacteria, the most common resistance mechanism is structural modification by bacterial enzymes; aminoglycoside phosphotransferases (APH), adenylyltransferases (AAD or ANT) and acetyltransferases (AAC). Typical sites of bacterial modification are exemplified (Figure 1) for kanamycin B, the aminoglycoside most susceptible to resistance.[5,15,16] A second mechanism of resistance is decreased uptake and/or accumulation of the drug in bacteria. Finally, streptomycin is susceptible to resistance by alteration of the ribosomal binding sites.[5]

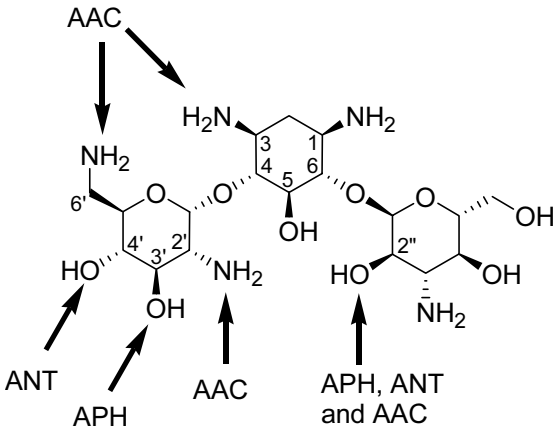


Figure 1. Major aminoglycoside-modifying enzymes acting on kanamycin B.[5]

Structural features

As the name aminoglycoside suggests, members of the aminoglycoside family are predominantly built up from a wide variety of aminosugars (Figure 2). These aminosugars, often of the 6-amino or the 2,6-diamino type, may be further decorated or functionalized, *e.g.* by deoxygenation, *N*- or *C*-methylation, or may contain additional stereocenters, and are always 1,2-*cis* glycosidically linked. Some additional rings or rare carbohydrates can also be identified in some aminoglycosides, but of utmost significance is a cyclohexitol present in each of the structures of Figure 2.

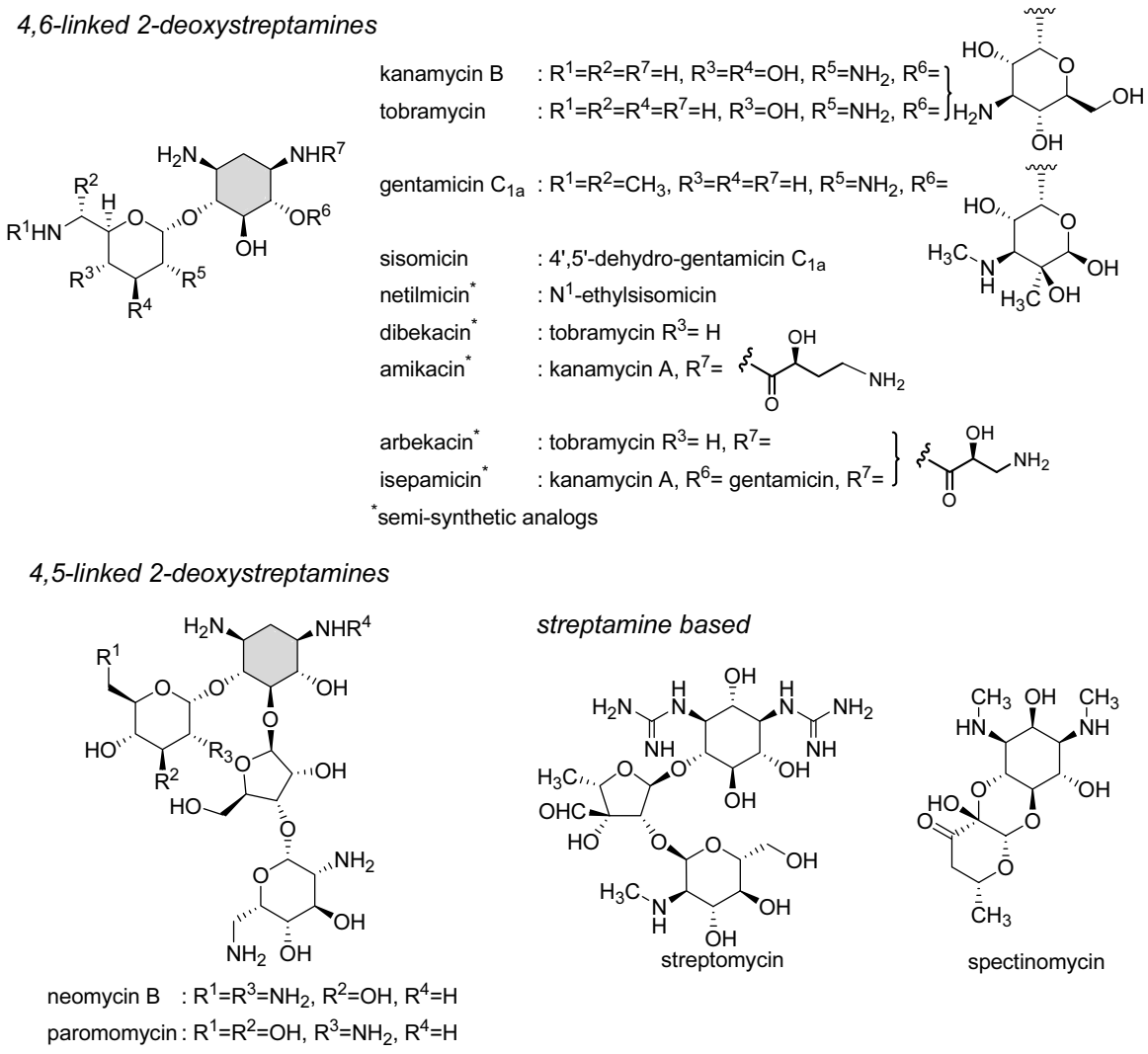


Figure 2.

This ring, termed streptamine or 2-deoxystreptamine (2-deoxy-*myo*-inosa-1,3-diamine or all-*trans*-1,3-diamino-4,5,6-trihydroxycyclohexane, 2-DOS for short), as highlighted in Figure 2, provides the central scaffold of any known aminoglycoside. It is an all-*trans* cyclohexitol, 1,3-substituted by two amino groups. Due to the presence of the plethora of amino functionalities, aminoglycosides are heavily protonated under physiological conditions, thus providing a rational for the strong affinity for (negatively charged) nucleotides.[17] Apart from the conserved 1,3-diamino functionality, the cyclohexitol contains three (2-DOS) or four (streptamine) hydroxyls, that provide the anchoring points for the aminosugars. Based on the substitution pattern of the streptamine ring, the aminoglycosides may be categorized in 4,5- or 4,6-linked. Although the streptamine numbering seems rather odd at first it is directly derived from its biogenic precursor *myo*-inositol (Figure 3) and is therefore numbered according to the method proposed by Fletcher *et al.* for inositols.[18] The same numbering is adapted in the case of 2-DOS (or 2-deoxy-*myo*-inosa-1,3-diamine), despite the fact that it is not biosynthetically derived from *myo*-inositol but from D-glucose-6-phosphate.

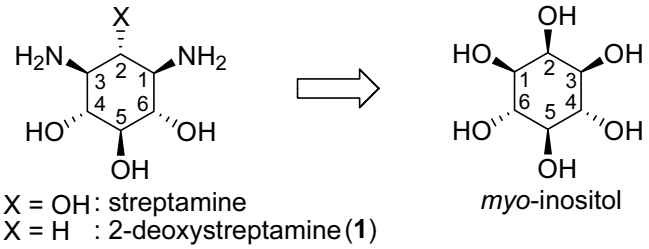


Figure 3.

Central scaffold of aminoglycosides: 2-DOS

The omnipresence and central location of 2-DOS in aminoglycoside structures suggests a pivotal role for biological activity, an intuitive assumption that is underlined by recently published X-ray crystal structures of several aminoglycosides complexed to the 30S ribosomal particle[19-22] or an A-site oligonucleotide sequence[23-25] which reveal without exception a similar binding pattern to 2-DOS, regardless of its 4,5- or 4,6-substitution. Recently X-ray crystal structures of paromomycin,[19,20,21] and hygromycin[22] complexed to the 30 S ribosomal subunit and of paromomycin,[23] tobraycin[24] and geneticin[25] complexed to oligonucleotides containing the minimal bacterial ribosomal A site are elucidated.[26] Apart from that, synthetic approaches toward novel aminoglycoside type RNA-ligands have also revealed a crucial role of 2-DOS for biological activity,[27] although some exceptions have been reported as well.[28,29] Convincing evidence of the strong affinity of 2-DOS for RNA was most recently provided by the observation that the bivalent structure of 2-DOS alone, that is without aminosugar substitution, already shows low micromolar binding to RNA hairpin loops.[30] An important reason therefore to synthesize 2-DOS is that by having the core structure in hand it should be possible to develop new and innovative antibiotics or, in a broader sense, RNA-binding molecules. From a chemical perspective, 2-DOS poses an interesting synthetic challenge due to the five contiguous stereogenic centers. In this respect, it is not surprising that numerous attempts have been made to obtain this aminocyclitol moiety synthetically and in this chapter a comprehensive overview of the synthetic routes that have appeared in literature will be provided.[31]

A summary of the synthetic efforts aimed at (derivatives of) 2-DOS (**1**) in terms of retrosynthetic analysis to starting materials, is provided in (Figure 1, first page of this chapter). It is interesting to note that the starting materials vary from simple cycloalkenes to highly oxygenated structures such as inositols and carbohydrates and in most cases already contain stereocenters. Keeping in mind that 2-DOS is a *meso* compound, the choice of a starting material from the chiral pool seems rather awkward at first sight, but in this manner a range of useful enantiomerically pure (protected) derivatives could be obtained (marked with an asterisk in Figure 1). A comprehensive

overview of the most important synthetic efforts will be provided in this chapter. Starting with the organic chemistry efforts, in three segments: (a) collection of meso 2-DOS and hyosamine (methyl-2-DOS) by degradation of neomycin (§**1.2**) or *via* synthetic routes (§**1.3**), (b) enantiomerically pure *protected* derivatives of meso 2-DOS, either by enzymatic resolution (§**1.4**) or from starting materials from the chiral pool (§**1.5**). Synthetic routes leading to regioisomers, dideoxyderivatives or syntheses of streptamine have also been reported, but are scarcer and are not included in this chapter.[31]

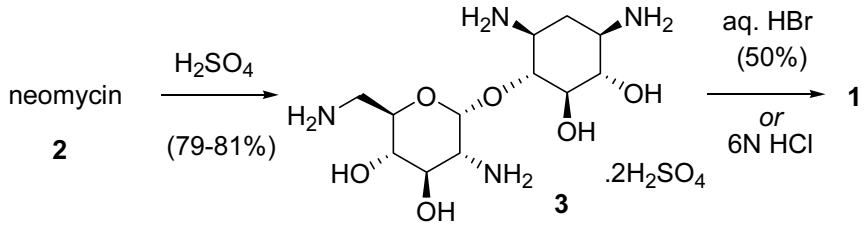
To date, the most practical method to obtain 2-DOS is by an acidic degradation of neomycin. One may wonder therefore: "What is the value of fully synthetic procedures that are inevitably more expensive and time-consuming?" The answer to this question lies in the fact that the "naked" compound obtained by hydrolysis of neomycin is as such useless for synthetic purposes. It demands extensive protective group manipulation but apart from that, since it is a *meso* compound, it also needs to undergo desymmetrization before incorporation in (enantiopure) aminoglycoside entities can be undertaken. For these reasons only, *de novo* synthesis from individually prepared components can already effectively compete with synthesis from neomycin. Apart from that, total synthesis has the obvious advantage that it not only allows full flexibility in design and preparation of novel aminoglycoside-type structures, but also opens up avenues leading to unnatural analogues.

1.2 Degradation of neomycin

Despite all efforts described later in this chapter, the simplest method to obtain 2-DOS still is by hydrolytic degradation of a natural aminoglycoside. In a strict sense, to obtain 2-DOS in this manner is not a synthesis, but since it is such a straightforward way to access 2-DOS it is by far the most popular route applied to date. The usual aminoglycoside of choice for this purpose is neomycin trisulfate (**2**), since it is readily obtained from *Streptomyces* fermentation,[32] and commercially available for a reasonable price (±0.6 €/mmol). Treatment of neomycin with sulfuric acid gives compound **3** by selective cleavage of a single glycosidic linkage (Scheme 1). This product, originally termed neomycin A as it was identified as a constituent of a mixture of three neomycins (A-C)[33] obtained from the bacterial broth, is now better known as neamine.[34]

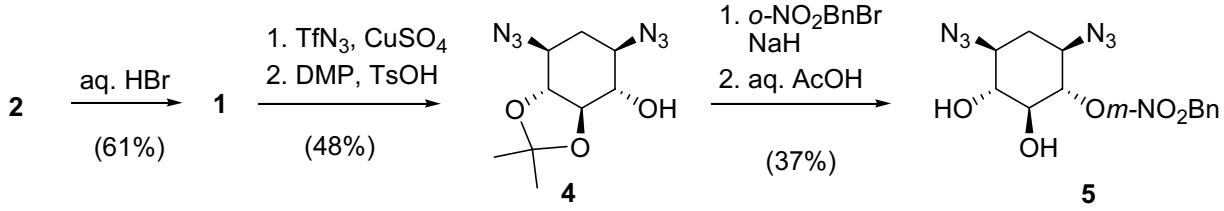
From neomycin B, two direct procedures to obtain (ammonium salts of) 2-DOS **1** have been described (Scheme 1), either by reaction of **2** with HBr (50% yield),[35] or by heating **2** in 6 N hydrochloric acid (yield not reported).[36]

Scheme 1.



In an alternative to these two-step procedures, 2-DOS (**1**) can also be directly obtained by complete hydrolysis of neomycin as first described by Georgiadis.[37] In order to separate **1** from the other carbohydrate constituents of neomycin, this route involves the isolation of **1** in a protected form. For example, selective *N*-protection of **1** with an acyl or urethane protective group is possible, but for spectroscopic and solubility reasons, it is most conveniently converted to a bisazide by diazotransfer. The latter transformation, first reported for neomycin by Alper *et al.*[38] converts both amines of 2-DOS directly to azides by a Cu^{2+} or Zn^{2+}-catalyzed diazotransfer (Scheme 2). As reported by Swayze *et al.*, [39,40,41] the resulting triol can be selectively blocked at *O*-4 and *O*-5 with an isopropylidene group to give **4** in racemic form. Finally, protection of the 6-position with a 3-nitrobenzyl group, followed by removal of the isopropylidene group leads to compound **5** in an overall yield of 11% for the five steps.

Scheme 2.



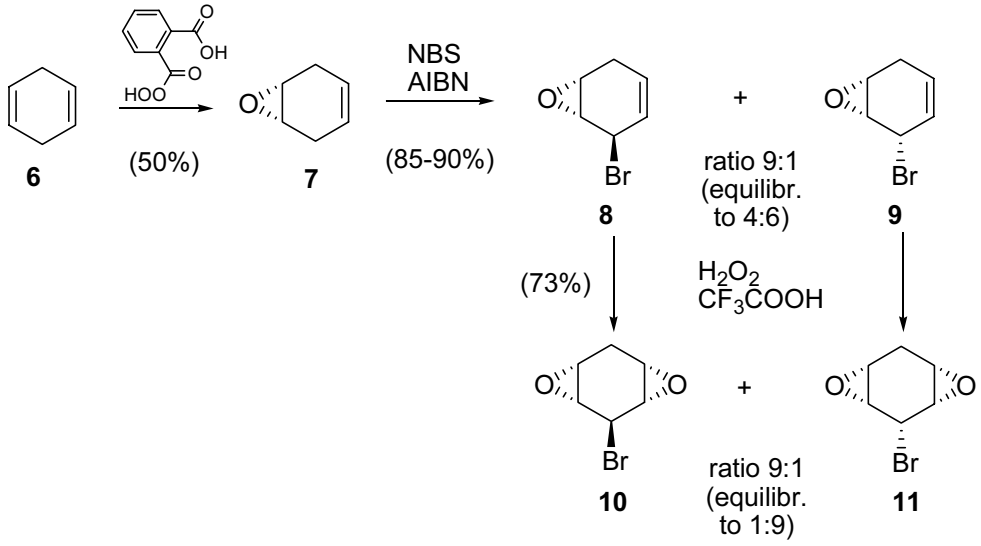
1.3 *De novo* synthesis of 2-DOS

It is obvious that a large variety of starting materials has been applied for the synthesis of 2-DOS, but only the most important synthetic routes leading toward 2-DOS will be discussed here. A comprehensive overview of syntheses of 2-DOS is given in a recent review.[31]

Prinzbach and coworkers[42,43,44] reported a synthesis of 2-DOS starting by epoxidation of 1,4-cyclohexadiene (**6**, Scheme 3) with monoperphthalic acid (**6**→**7**).[45] Subsequent allylic bromination with *N*-bromosuccinimide in CCl_4 gives a 9:1 mixture of (the rather explosive) compounds *trans*-**8** and *cis*-**9**. Although it was possible to equilibrate the latter mixture to a more desirable ~4:6 ratio upon the action of tetrabutylammonium bromide in MeCN (or acetone), a more desirable route involved *cis*-diastereoselective epoxidation of the crude mixture of **8** and **9** with trifluoroperacetic acid. It was found that the resulting 9:1 mixture of diepoxides **10** and **11** was also

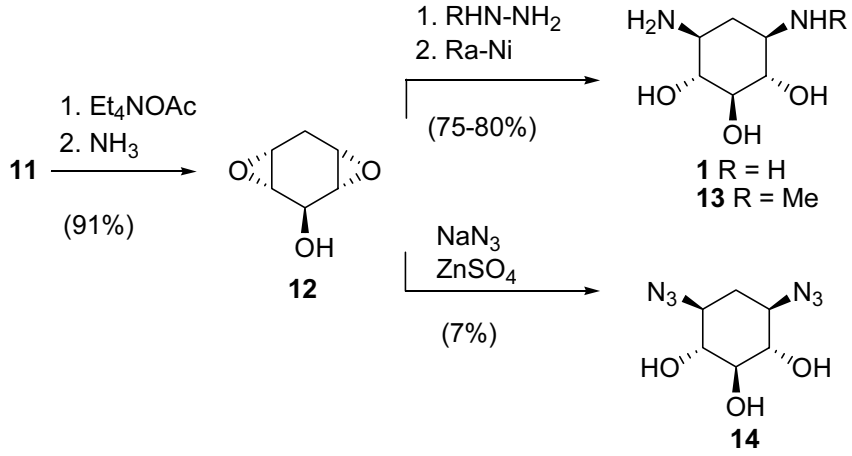
amenable for equilibration under the influence of an ammonium bromide in acetone, to give the mirror 1:9 ratio of **10** to **11**.[46] The desired all-*cis*-diepoxide **11** could thus be obtained in pure form after selective crystallization from the mixture in methanol.

Scheme 3.



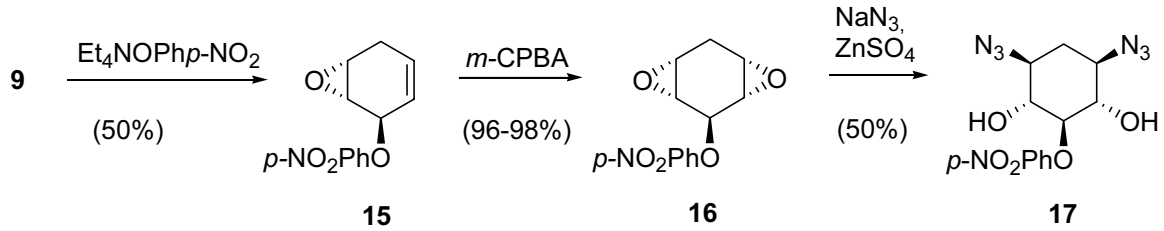
Having the *cis*-diepoxide **11** in hand three alternative routes were described to synthesize 2-DOS. Firstly, treatment of **11** with tetraethyl ammonium acetate in acetone led to nucleophilic substitution of the bromide and after subsequent deacetylation, alcohol **12** (Scheme 4). Application of Suami's hydrazinolysis/hydrogenation procedure gave aminocyclitol **1** in 75-80% yield.[31] The total yield of compound **1** starting from 1,4-cyclohexadiene in 7 steps is approximately 24%. Similarly, subjection of **12** to methylhydrazine led to (±)-hyosamine (**13**), the *N*-methyl derivative of 2-DOS, in a yield of approximately 70%. The diazidocyclitol **14** could also be obtained from **12** by reaction with sodium azide but in this case the overall yield is only 2.1% for the 6 steps.

Scheme 4.



A third variation on the theme provided by the same research group involves conversion of monoepoxide **9** to the *p*-nitrophenylether **15** with tetraethylammonium 4-nitrophenolate (Scheme 5). Subsequent stereoselective epoxidation to diepoxide **16** followed by opening of both epoxides with sodium azide led to a much improved yield of 50% and an overall yield of the protected 2-DOS derivative **17** in 5 steps and 6.6%. Final removal of the *p*-nitrophenyl group was not reported.

Scheme 5.

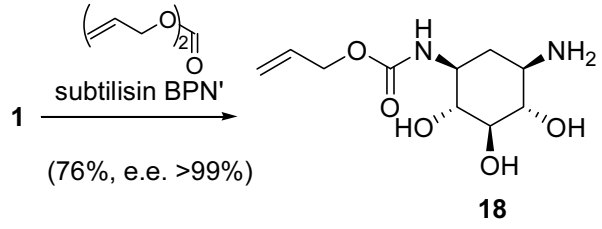


1.4 Desymmetrization

None of the syntheses of 2-DOS discussed above led to enantiopure material. The reason for this is that despite its five stereocenters, 2-DOS (or any symmetrically protected variant of it) is a meso compound, due to the presence of an internal plane of symmetry (although an unlikely optical rotation of 41.8° has been reported).[37] Obviously, if synthesis of 2-DOS itself is the prime goal, molecular symmetry makes life easier. However, if 2-DOS is to serve as a scaffold for incorporation in an enantiopure aminoglycoside (analogue), a desymmetrized variant is a prerequisite.

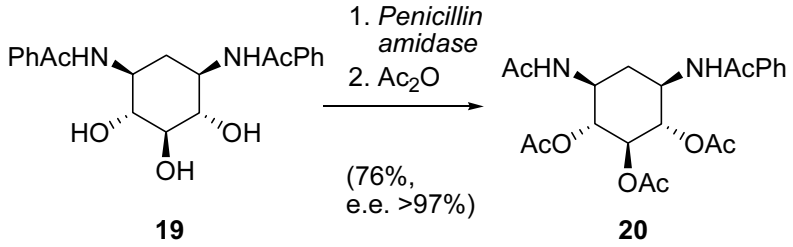
A most convenient approach toward an enantiopure derivative of 2-DOS, since it is so readily obtained by hydrolysis of neomycin, involves resolution. Nevertheless, the first (enzymatic) resolution of 2-DOS was not reported until 1996, by Orsat *et al.*,[47] and involved the conversion of meso 2-DOS **1** into **18** through the combined action of the catalase subtilisin BPN' and diallylcarbonate in HEPES buffer (Scheme 6). The product (**18**) was obtained (1 week, room temperature) in a yield of 76% and with excellent selectivity (>99% e.e.).

Scheme 6.



Nearly at the same time, another example of 2-DOS resolution was provided by incubation of a suspension of *N*,*N'*-di(phenylacetyl) protected 2-DOS (**19**) in a 4:1 phosphate buffer: DMF mixture (18 days, 25-35 °C) with *Penicillin amidase* to give, after acetylation, the *N*-acetyl,*N'*-phenylacetyl protected derivative **20** with high enantioselectivity (Scheme 7).[48]

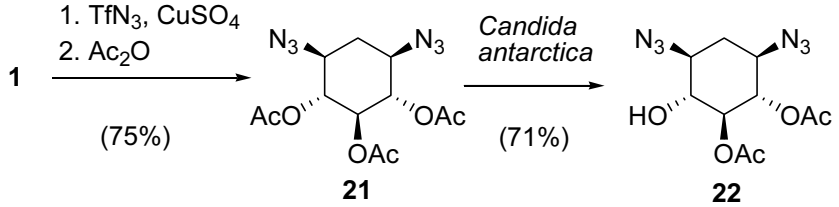
Scheme 7.



19 **20**

More recently, Wong *et al*. described the desymmetrization of a diazido derivative of 2-DOS by either an enzymatic or a chemical approach.[49] Thus, 2-DOS was converted into the diazido triacetyl derivative (**21**) by a Cu^{2+}-catalyzed diazotransfer with triflyl azide, followed by treatment with acetic anhydride (Scheme 8). Enzymatic resolution of **21** relied on enantioselective deacetylation using a resin-immobilized lipase Novozym 435 (immobilized *Candida antarctica*), providing **22** in 71% yield (although the e.e. was not mentioned).

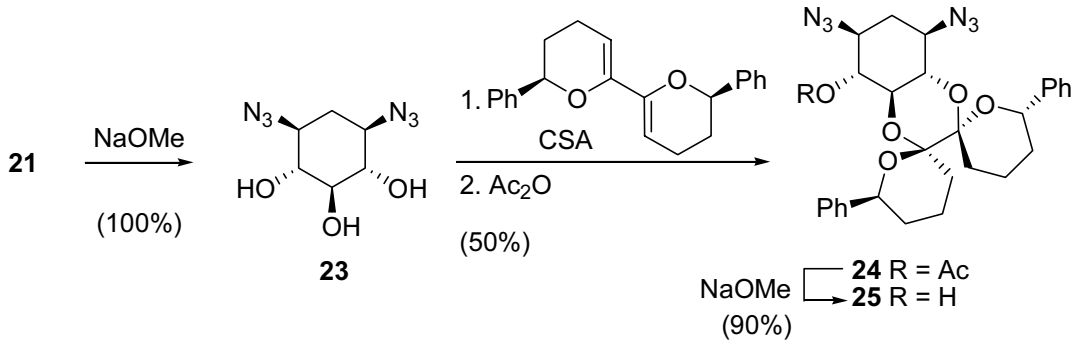
Scheme 8.



21 **22**

An alternative chemical desymmetrization approach utilized the chiral dispiroketal protection-desymmetrization protocol of Ley and coworkers.[50] After deacetylation of **21**, the resulting triol **23** was treated with (2*R*,2*R'*)-bis-diphenyl-6,6'-bis(3,4-dihydro-2*H*-pyran, PDHP) and catalytic camphorsulfonic acid in refluxing chloroform, followed by acetylation to give **24** as the major isomer in 50% (Scheme 9). Deacetylation provided **25**, a suitable substrate for selective glycosylation at the 4-position in a dedicated synthesis of aminoglycosides, but **25** was found to be of little value as carbohydrate acceptor due the inreactivity of the (sterically hindered) free hydroxyl.

Scheme 9.

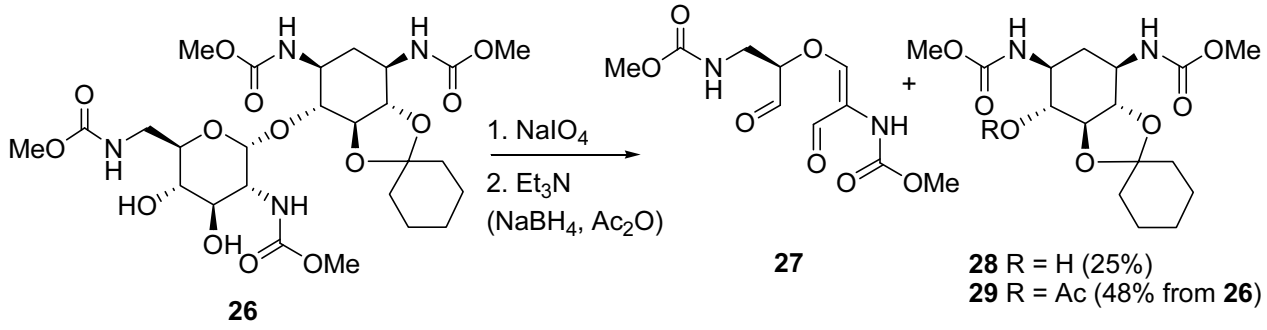


1.5 Enantiopure 2-DOS (derivatives) from the chiral pool

Apart from strategies employing resolution, a large number of synthetic routes toward enantiopure 2-DOS (derivatives) that have appeared in the literature are based on chiral starting materials such as neomycin, *N*-acetyl-D-glucosamine, D-mannose,[58] and D-glucose.[54]

Canas-Rodriguez reported the first synthesis of an asymmetric (protected) form of 2-DOS starting from neamine (**3**).[51] Oxidative cleavage of compound **26** with sodium periodate followed by E2-elimination upon treatment of the resulting dialdehyde with triethylamine gave **28** (Scheme 10). For reasons of purification the crude reaction mixture was subsequently treated with sodium borohydride after which alcohol **28** could be isolated in 25% yield or, preferably, as the acetate **29**. The overall yields starting from neomycin B are 8.7% and 17% for compounds **28** and **29**, respectively. We have adapted this methodology for the preparation of more conveniently protected *N*-Cbz protected 2-DOS (not depicted) and found the procedure works just as well.[52]

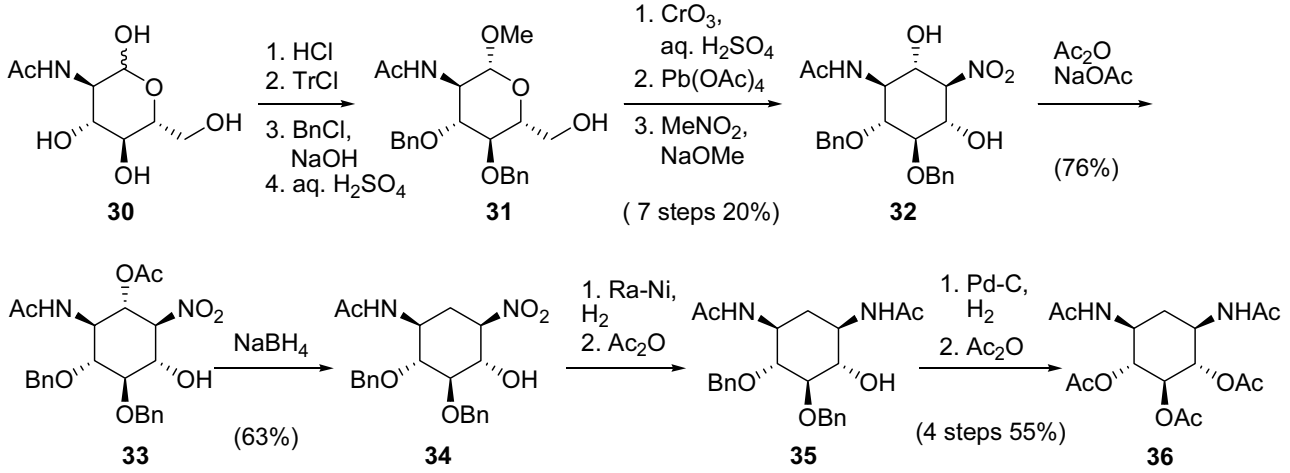
Scheme 10.



Yoshikawa designed a route toward 2-DOS centered on nitro-aldol reaction and a one-step elimination-reduction sequence of an acetoxy residue (Scheme 11).[53] To this end, Fisher glycosidation of *N*-acetyl-D-glucosamine (**30**) was followed by selective protection of the primary hydroxyl by a trityl group, benzylation and acidic detritylation to give **31**. Subsequent Jones oxidation with CrO_3 with concomitant hydrolysis at the anomeric center, $Pb(OAc)_4$ cleavage of the masked glycol at O5, O6 followed by Henry reaction of the intermediate dialdehyde with

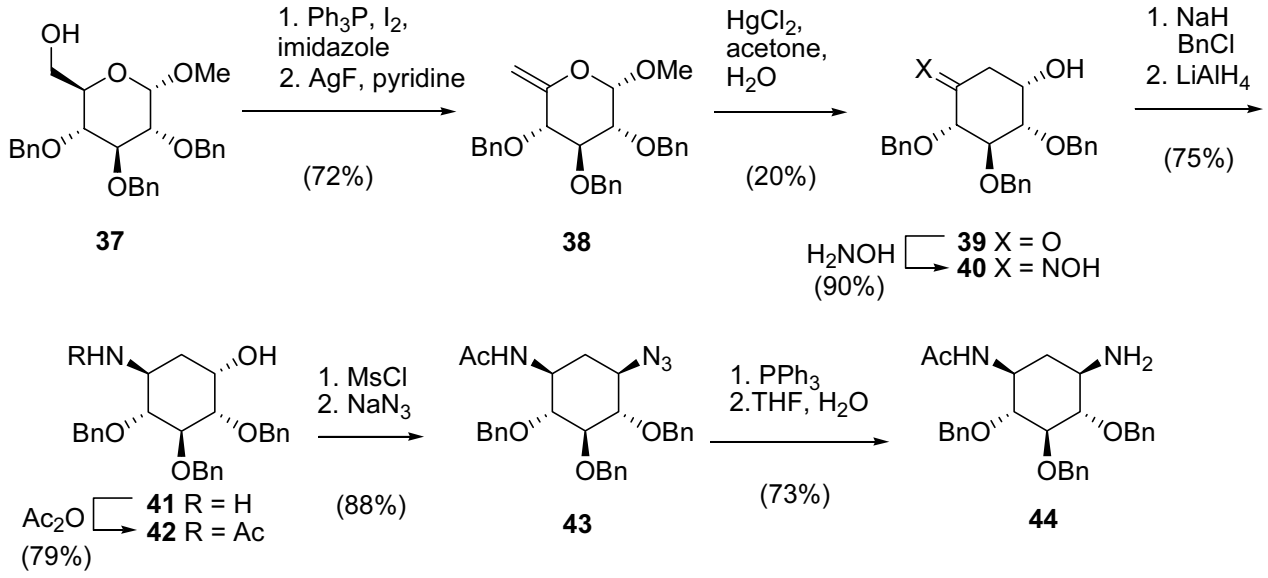
nitromethane gave the all-equatorial **32** with remarkable stereoselectivity. Selective protection of the 1,3-hydroxylamide using Ac_2O-NaOAc in THF was followed by $NaBH_4$-induced elimination of the acetoxy group and reduction leading to nitroaminocyclitol **34**. The overall yield of these 9 steps is 9.6%. The configurational identity of **34** was unambiguously established via reduction of the nitro group, *N*-acetylation, debenzylation and *O*-acetylation to afford the known 2-DOS pentaacetate **36**.

Scheme 11.



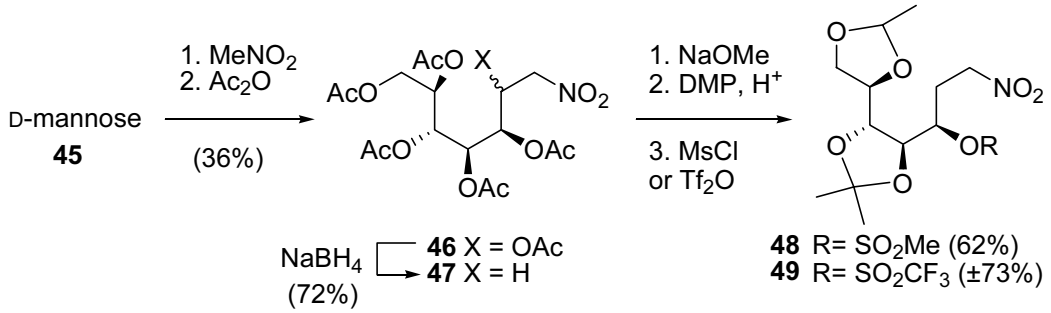
Da Silva *et al.* developed a route toward 2-DOS which appears to be inspired by the biosynthetic pathway of 2-DOS from D-glucose.[54,55] Oxime **40** was obtained from the α-methyl glycoside **37** *via* the carbohydrate-inose Ferrier rearrangement (**38**→**39**, Scheme 12).[56,57] Direct reduction of oxime **40** with $LiAlH_4$ was unsuccessful but benzylation of the oximino group prior to reduction led to a mixture of epimeric amines along with the *O*-benzylhydroxylamine (not depicted) in a 90% combined yield (ratio 1:9:1). Acetylation of the desired *trans*-epimer **42** followed by conversion to the mesylate and reaction with sodium azide led to protected azido 2-DOS **43**. Staudinger reaction with triphenylphosphine gave the protected 2-DOS derivative (**44**) in an overall yield of 4.9% starting from protected methyl glycoside **37**.

Scheme 12.



Baer and coworkers[58] developed two (similar) routes toward enantiopure 2-DOS analogues, both starting from D-mannose (**45**, Scheme 13). Via a known Henry reaction, D-mannose was first converted into 1-deoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol hexaacetate (**46**).[59] Elimination-reduction with sodium borohydride followed by *O*-deacetylation gave the pentanol (not depicted) which was regioselectively acetonated. Mesylation or triflation afforded the sulfonates **48** and **49** in an overall yield from D-mannose of 20-25%.

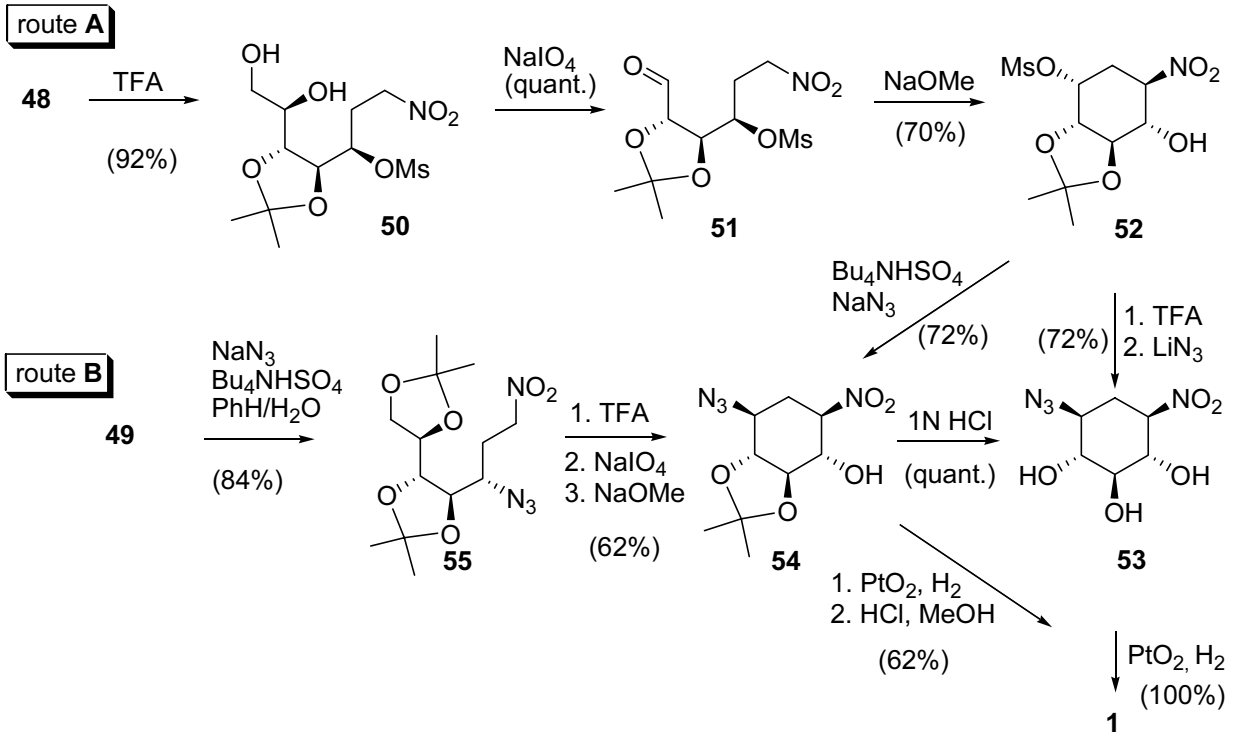
Scheme 13.



Compounds **48** and **49** were the starting materials for two alternative routes. First of all, following route A (Scheme 14), mesylate **48** was selectively deacetonated at the 6,7-position with trifluoroacetic acid and subsequently cleaved with sodium periodate to afford compound **51**. Nitroaldol cyclization of the sugar effected by sodium methoxide in methanol led to a mixture of epimeric cyclitols that could be separated by chromatography to give predominantly diastereomer **52** (70% yield). Conversion of **52** into **53** could be executed by two routes, either involving deacetonation with TFA prior to nucleophilic introduction of an azide (72% for 2 steps), or by reaction of **52** with sodium azide under phase transfer catalysis in benzene/water (**52→54**) followed

by acid hydrolysis (72% yield). Unfortunately, in both cases nucleophilic displacement was accompanied by partial epimerization at the carbinol positions, necessitating tedious chromatographic separation. Final hydrogenolysis of **53** with Adam's catalyst to 2-DOS was straightforward. The alternative route B involves the preparation of azido derivative **55** from triflate **49** prior to Henry-aldol cyclization. In similar steps as above, nitro alcohol **54** was obtained, along with its epimer, in 62% overall yield (**54** was isolated by crystallization performed under dynamic epimerization conditions). An alternative route from **54** to **1** was also presented involving hydrogenation to the 4,5-isopropylidene protected 2-DOS **54** prior to deacetonation. The total yield for both routes starting from D-mannose is around 13%. It is of interest to note that the enantiopure alcohol **54** is potentially suitable for the preparation of aminoglycoside analogues by *O*-glycosylation.

Scheme 14.



1.6 Conclusion

An efficient route to 2-DOS may serve as a practical starting point for the assembly of aminoglycoside-type libraries in the development of novel RNA-ligands. As such, the syntheses discussed in this chapter are not of sole academic interest since they lead to a valuable starting material for biological and medicinal purposes. It is apparent, however, that the above described synthetic sequences aiming at 2-DOS are characterized by a wide variety of strategies. Apart from that, nearly each route starts from its own unique starting material (with the exception of neomycin)

and, more importantly, leads to a unique 2-DOS analogue, rather than 2-DOS itself, in most of the cases. A few observations can be readily made. First of all, by far the highest overall yield to acquire 'naked' 2-DOS is by degradation of the relatively cheap and commercially available aminoglycoside neomycin. Although the synthetic elegance of the approach is poor, with 75% of starting material degraded to waste, the ease and straightforwardness of the procedure outperforms any of the *de novo* syntheses. Therefore, if plain 2-DOS is required, acid hydrolysis of neomycin obviously is the method of choice (apart from direct acquisition of 2-DOS). The syntheses shown in this chapter start from a large diversity in starting material, both in structure and in price. However, for cost-effective synthesis of 2-DOS, or an analogue thereof, the price of the starting material is only one of the factors to be taken into consideration. Apart from that, the number of synthetic steps, costs of reagents and purification, intermediate yields and applicability to large scale are only some of the additional factors that define the value of a synthetic sequence. Obviously, 'naked' 2-DOS or a symmetrically protected variant thereof can be readily obtained, but the practicality of these *meso* compounds (or any other analogue) depends entirely on an individual's particular goal.

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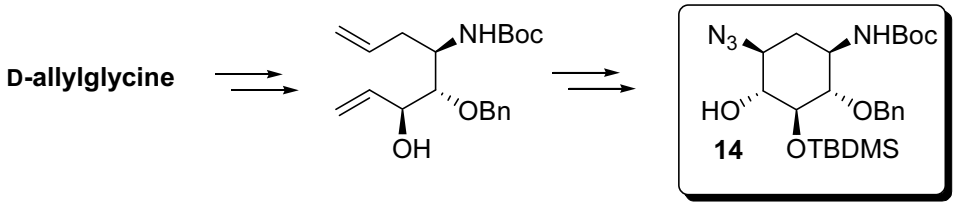
2 Synthesis of a protected enantiomerically pure 2-deoxystreptamine derivative from D-allylglycine[1]

Abstract

A diastereoselective synthetic route from D-allylglycine to the enantiopure (protected) 2-deoxystreptamine (2-DOS) derivative **14** is presented. Key steps involve two consecutive chain extensions with crucial stereodirective roles for the amino protective groups, as well as ring closure by olefin metathesis, face selective dihydroxylation, cyclic sulfate formation and finally opening with azide. The resulting 2-DOS derivative is ideally protected for the preparation of 4,5- or 4,6-linked aminoglycoside antibiotics.



[1] Part of this chapter has been published in: G. F. Busscher, F. P. J. T. Rutjes, F. L. van Delft, *Tetrahedron Let.* **2004**, *45*, 3629-3632.

2.1 Introduction

Since the discovery of streptomycin in 1944, the family of aminoglycosides has grown steadily into a powerful class of antibiotics with a broad antibacterial spectrum and proven efficacy, particularly in combination with other drugs.[1] Nevertheless, extensive use of the aminoglycosides is limited due to the associated toxicities, most notably nephrotoxicity and ototoxicity, and to a lesser extent neuromuscular blockade.[2] Another, more alarming drawback of the aminoglycosides is the global development of microbial resistance with the most common mechanism being structural modification by bacterial enzymes. These circumstances necessitate the development of new and innovative aminoglycoside antibiotics and several reports on the derivatization of existing aminoglycosides have appeared in the literature in recent years.[3] To be fully flexible in the design and preparation of novel aminoglycoside-type structures, however, *de novo* synthesis from individually prepared components is required. Consequently, our research has focused on the synthesis of enantiomerically pure derivatives of 2-deoxystreptamine (2-DOS), an aminocyclitol ring that constitutes the core structure of the majority of clinically useful aminoglycosides (Figure 1) and, as may well be speculated, is crucial for binding of the aminoglycosides to their target A-site RNA.[4]

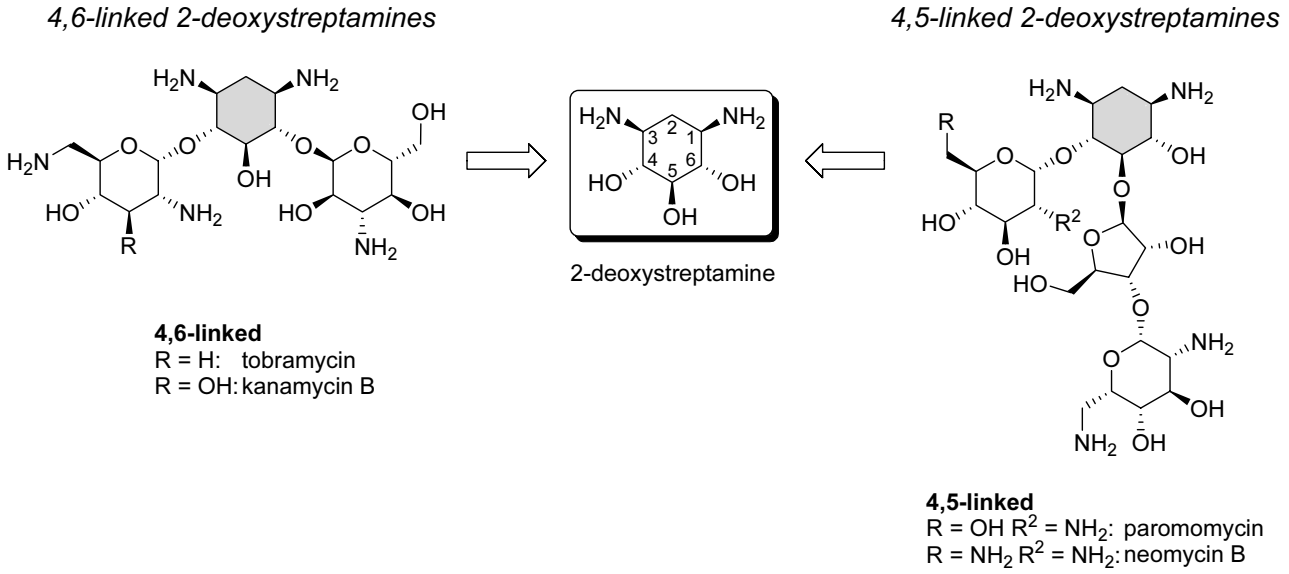


Figure 1. Representative aminoglycoside antibiotics, both 4,5- and 4,6-linked to 2-DOS.

Although several synthetic routes towards 2-DOS are known in the literature, all require many synthetic steps and offer minimal flexibility in protective groups.[5] Moreover, only two of these routes afford an asymmetric analogue of 2-DOS, starting from either D-mannose[6] or D-glucose.[7] The most practical method to obtain this aminocyclitol moiety is by degradation of neomycin,[8,9,10]

but the "naked" *meso*-compound thus obtained still requires desymmetrization as well as protective group manipulations before incorporation into new aminoglycoside entities can be ensured. In contrast, we here wish to report a synthesis of an orthogonally protected, enantiopure 2-DOS derivative which is highly suitable to serve as a scaffold for new aminoglycoside entities, either 4,5- or 4,6-linked.

It was envisioned that the final protected 2-DOS derivative **A** could be obtained from the cyclohexane precursor **B** *via* epoxidation and nucleophilic ring opening. Compound **B** in turn can be retrosynthetically traced back via ring closing metathesis and vinyl addition to the masked aldehyde **C**. The latter thiazole-protected *syn* β-amino alcohol **C** was expected to result in a few steps from D-allylglycine.

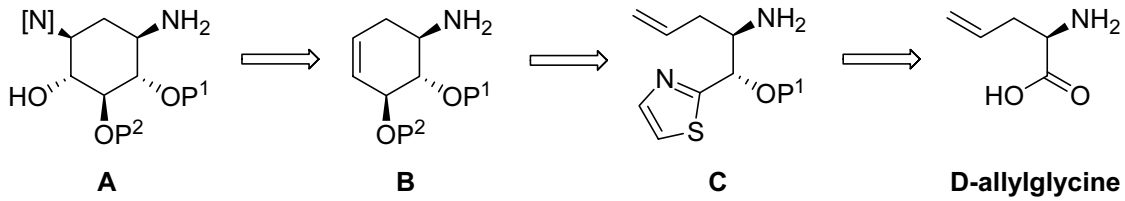
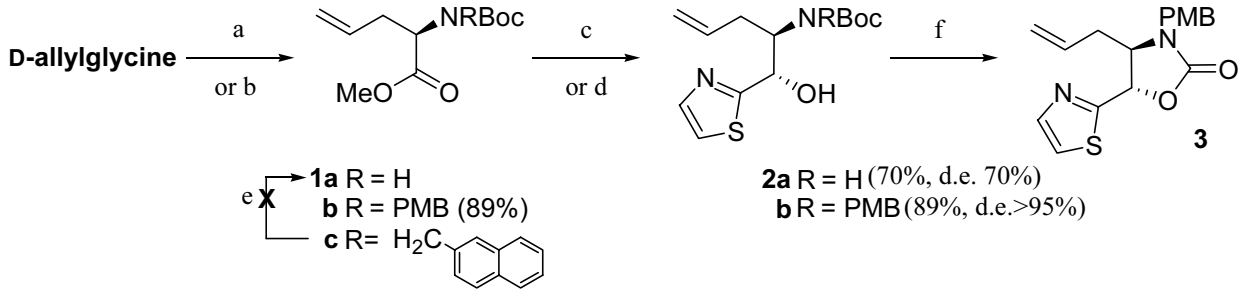


Figure 2. Retro-synthetic analysis of protected 2-DOS

Our synthesis starts from enantiomerically pure D-allylglycine, a non-proteinogenic amino acid that is readily available in our group.[11] Thus, the methyl ester of D-allylglycine[12] was subjected to conditions explored by Dondoni and co-workers,13 involving introduction of thiazole as a masked aldehyde. Our first attempt to obtain the thiazolyl β-amino alcohol **2a** (Scheme 1) started with Boc-protected allylglycine methyl ester **1a**, which gave upon partial reduction and addition of 2-(trimethylsilyl)thiazole (2-TST) the *syn*-amino alcohol **2a**. Not unexpectedly, however, alcohol **2a** was obtained13 with a d.e. of only 70% which led us to follow a slightly different procedure involving double protection of the amino function (Boc, PMB) as in **1b** and reversal of the sequence of events, *e.g.* first reaction with 2-lithiothiazole (2-LTT) and then $NaBH_4$ reduction of the resulting ketone. To our satisfaction, the *syn* β-amino alcohol **2b** was now obtained with a d.e. of >95%.[13]

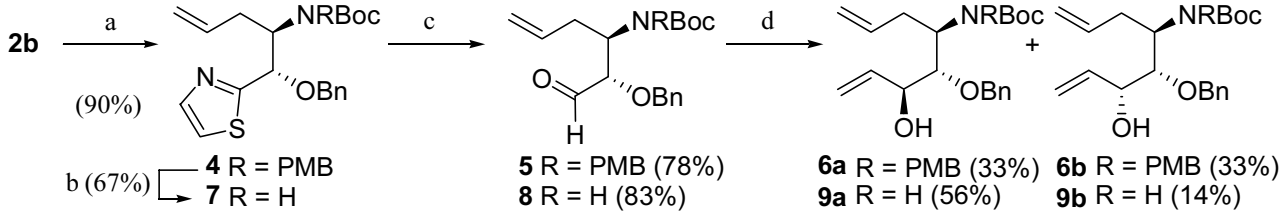
Scheme 1.



Reagents and conditions: (a) AcCl, MeOH, 2 h, Δ then Boc_2O, dioxane, rt, 18 h (90%); (b) AcCl, MeOH, Δ, 2 h then Et_3N, *p*-$MeOC_6H_4CHO$ or naphtylaldehyde, CH_2Cl_2, rt, 18 h then $NaBH_4$, MeOH, 30 min, 0 °C then Boc_2O, dioxane, rt, 18 h (**1b** 89%, **1c** 60%); (c) DIBAL-H, toluene, -78 °C, 2 h, 2-TST, CH_2Cl_2, -20 °C, 48 h then $Bu_4NF.3H_2O$, THF, rt, 1 h (70% d.e., 70%); (d) 2-LTT, Et_2O, -78 °C to -45 °C, 6 h then $NaBH_4$, MeOH, 0 °C, 30 min; (e) DDQ, CH_2Cl_2/MeOH (4/1), drop H_2O, rt, o.n.; (f) NaH, DMF, 0 °C.

Next, benzyl protection of the free hydroxyl was investigated but it was found that standard conditions (sodium hydride followed by benzyl bromide) led to 2-oxazolidinone **3** (Scheme 1). However, inverting the order of addition of NaH and BnBr cleanly gave the *O*-benzylated derivative **4** (Scheme 2) which was subjected to the thiazole deblocking protocol,[13] followed by condensation of the resulting aldehyde **5** with vinylmagnesium bromide. Unfortunately, the nucleophilic chain extension was characterized by low stereoselectivity revealing a 1:1 mixture of *syn* and *anti*-diastereomers **6a** and **6b** which did not improve upon varying the reaction conditions (temperature, solvents, additives).

Scheme 2.

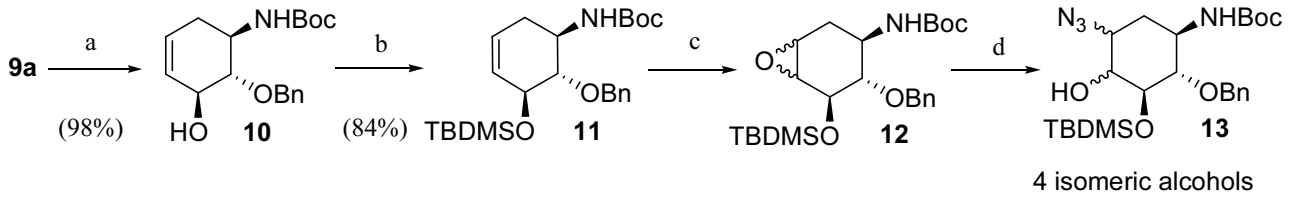


Reagents and conditions: (a) BnBr, DMF, 0 °C then NaH, 1 h; (b) CAN, $NaHCO_3$, MeCN/H_2O (4/1), rt, 30 min; (c) 4Å MS, MeOTf, MeCN, rt, 25 min then $NaBH_4$, MeOH, 0 °C, 10 min then $CuCl_2.2H_2O$, CuO, MeCN/H_2O, 10/1, rt, 15 min; (d) vinylMgBr, THF, -78 °C, 4 h.

A crucial role of the amino protection was again suspected and therefore it was decided to remove the *p*-methoxybenzyl group of **4** (→**7**) with CAN prior to Grignard addition. Buffering of the CAN solution with $NaHCO_3$ was required to prevent a drop in yield, the monoprotected amine **7** was now obtained in a yield of 67%. Attempts to increase the yield, for instance via deprotection with DDQ, were not fortunate since only starting material was recovered. A route proceeding via the naphtylmethyl protected urethane **1c,** based on the premise that a naphtylmethyl group could be removed easier was also not successful due to the fact we were unable to remove the naphthylmethyl group with DDQ (Scheme 1).[14] Nevertheless, having the monoprotected urethane **7**

in hand, addition of vinylmagnesium bromide after thiazole unmasking (**7→8**) could be attempted. Gratifyingly, the diastereoselectivity of the addition now proceeded with a much improved *syn*:*anti* ratio of 4:1, leading to compounds **9a** and **9b**, respectively. (Scheme 2).

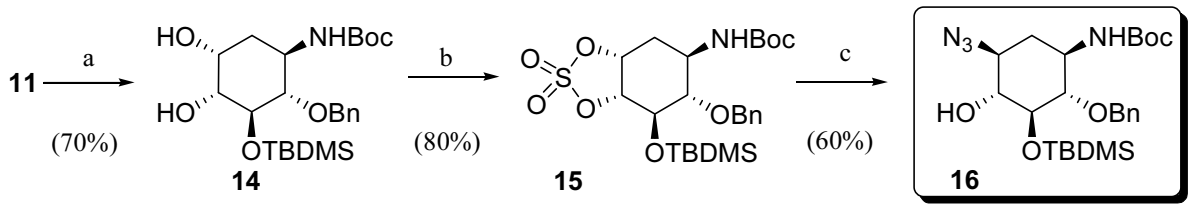
Scheme 3.



Reagents and conditions: (a) 2nd generation Grubbs' catalyst (mono-substituted imidazolylidene ligand), CH_2Cl_2, rt, 2 h; (b) TBDMSOTf, Et_3N, CH_2Cl_2, rt, 2 h; (c) several methods *e.g.* *m*-CPBA, CH_2Cl_2, rt, 48 h, rt or oxone, chlorocyclohexanone, $NaHCO_3$, $MeCN/H_2O$ 3:2, 24 h; (d) NaN_3, NH_4Cl, MeOH.

After silica gel separation of the diastereomers, ring-closing metathesis proceeded smoothly and the free hydroxyl of the cyclic product **10**[15] was protected with a TBDMS group to afford **11** in 84% yield (Scheme 3). Despite the presence of the bulky silyl group, however, epoxidation of compound **11** with several reagents, such as *m*-CPBA, oxone and *in situ* formed dioxirane[16] led in all cases to inseparable mixtures of diastereomers varying from 1:1 to 3:1 (**12**). Matters became more complicated when we tried to open the diastereomeric epoxides with sodium azide, leading to the formation of four isomeric azido alcohols (**13**).

Scheme 4.



Reagents and conditions: (a) $RuCl_3.H_2O$, $NaIO_4$, $EtOAc/MeCN/H_2O$ (3/3/1), 0 °C, 3 min; (b) $SOCl_2$, pyridine, EtOAc, 0 °C, 30 min then $NaIO_4$, $RuCl_3.H_2O$, $CH_2Cl_2/MeCN/H_2O$ (2/2/3), 0 °C, 1 h; (c) LiN_3, DMF, Δ, 4 h then H_2SO_4, THF, H_2O, rt, 30 min.

Therefore, attention was focused on the application of cyclic sulfate technology, since it is known that the reactivity of cyclic sulfates and epoxides towards nucleophiles is similar in nature but differs in selectivity.[17] Another advantage is that the ring-opening of five-membered cyclic sulfates proceeds much faster than with epoxides probably due to the better leaving group ability.[18] Thus, the double bond of **11** was dihydroxylated[19] (**11→14**, Scheme 3), which occurred with exclusive facial selectivity, followed by reaction with thionyl chloride and oxidation to form the cyclic sulfate **15** in 80% yield.[20] Much to our satisfaction, opening of the cyclic sulfate with lithium azide proceeded completely regioselectively to give, after subsequent sulfate hydrolysis, the protected

2-DOS **16** in enantiomerically pure form. The latter compound, after glycosylation of the free hydroxyl, is ideally suited for the preparation of either 4,5- or 4,6-linked aminoglycoside analogues after subsequent desilylation or debenzylation, respectively.

2.2 Concluding remarks

In conclusion, we believe that the synthesis described above is a versatile route towards orthogonally protected 2-DOS in enantiomerically pure form in 14 steps and an overall yield of 6.1%. The aminocyclitol building block obtained is suitable for incorporation into new aminoglycoside entities.

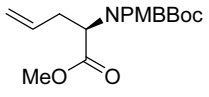
2.3 Acknowledgements

K. Koch and C. Schortinghuis are gratefully acknowledged for the HPLC measurements. Dr. R. Blaauw (Chiralix B.V., Nijmegen, The Netherlands) is gratefully acknowledged for providing D-allylglycine.

2.4 Experimental Section

General methods

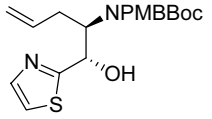
All reactions were carried out under inert atmosphere of dry nitrogen or argon. Standard syringe techniques were applied to the transfer of dry solvents and air- or moisture sensitive reagents. R_f values are obtained using thin layer chromatography (TLC) on silica gel-coated plates (Merck 60 F254) with the indicated solvent mixture and compounds were detected with UV light, ammonium molybdate solution, potassium permanganate, ninhydrin, or with anisaldehyde. Melting points were analyzed with a Büchi melting point B-545. IR spectra were recorded on an ATI Mattson Genesis Series FTIR spectrometer; absorption reported in cm-1. GC was performed on a Hewlett Packard 5890, containing a HP1 column (25 m * 0.32 mm * 0.17 μm), FID detection, and equipped with a HP3393A integrator. NMR spectra were recorded on a Bruker DMX 300 (300 MHz), and a Varian 400 (400 MHz) spectrometer in $CDCl_3$ solutions (unless otherwise reported) using TMS as internal standard; chemical shifts are given in ppm. Coupling constants are reported as *J*-values in Hz. Peak assignment in ^{13}C spectra are based on 2D-GHSQC and GHMBC spectra. Enantiomeric purities were determined on a Shimadzu HPLC, with indicated column and solvent mixture. Column or flash chromatography was carried out using ACROS silica gel (0.035–0.070 mm, and ca 6 nm pore diameter). Automated parallel syntheses were performed with the Anachem synthesis robot (Gilson SK233). Optical rotations were determined with a Perkin Elmer 241 polarimeter. Mass spectra were measured with a Fisons (VG) Micromass 7070E apparatus and/or a Finnigan MAT900S. MALDI-TOF-MS spectra were measured on a Bruker Biflex III machine, with dihydroxybenzoic acid (DHB) as matrix. Elemental analyses were carried out using a Carlo Erba Instruments CHNS-O EA 1108 element analyzer. Solvents were distilled from appropriate drying agents prior to use. Unless stated otherwise, all chemicals were purchased and used as such. The microwave reactions were carried out in a CEM Discover microwave.



(4*R*)-*N*-(*tert*-Butoxycarbonyl)-*N*-(4-methoxybenzyl)allylglycinemethylester (1b)

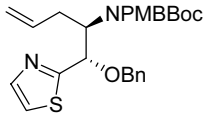
AcCl (18 mL) was added dropwise to cold MeOH (ice bath) (118 mL). The reaction mixture was stirred for 5 min before D-allylglycine was added (10.0 g, 87.0 mmol). After refluxing for two hours the reaction mixture was concentrated. The obtained allylglycine methylester (5.0 g, 25 mmol) was dissolved in CH_2Cl_2 (200 mL) under an argon atmosphere. To this solution was added *p*-methoxybenzaldehyde (5.60 mL. 104 mmol), Et_3N (6.50 mL, 104 mmol) and $MgSO_4$ (6.00 g). The

reaction mixture was stirred for 18 hours and filtered through celite and evaporated. The crude mixture was dissolved in MeOH and cooled with an ice bath. $NaBH_4$ (1.90 g, 50.0 mmol) was added and the reaction mixture was stirred for 30 min. After the addition of acetone solvents were evaporated. The reaction mixture was washed with sat. aq. $NaHCO_3$, brine and extracted with EtOAc, and dried with Na_2SO_4, the crude product was dissolved in dioxane (40 mL) and di-*tert*-butyldicarbonate (6.00 g, 104 mmol) was added to the reaction mixture. After stirring for another 18 hours the solvents were evaporated and the crude product was purified by flash chromatography (EtOAc/*n*-heptane, 1/5), to obtain **1b** (13.0 g, 89%, 3 steps) as a colorless oil. R_f 0.7 (EtOAc/*n*-heptane, 3/2). $[\alpha]_D^{20}$ +31 (c 0.68; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 2979, 1732, 1496, 1365, 1167, 1052. ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.22 (br s, 2H), (d, 2H), 5.78-5.51 (m, 1H), 5.15-4.89 (m, 2H), 4.78-4.29 (m, 2H), 3.90-3.71 (m, 1H), 3.80 (s, 3H), 2.60 (s, 3H), 2.89-2.62 (m, 1H), 2.61-38 (m, 1H), 1.55 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 130.5, 118.0, 114.3, 114.0, 70.2, 69.7, 55.9, 52.5, 38.2, 29.1, 29.0. HRMS (CI) *m/z* calcd for $C_{19}H_{27}O_5N$ $(M)^+$: 350.1967, found: 350.1967.



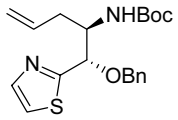
(1*R*,2*S*)-2-[2-*N*-(*tert*-Butoxycarbonyl)-*N*-(4-methoxybenzyl)amino]-1-hydroxy-3-allylpropyl]-1,3-thiazole (2b)

To a cold (-78 °C) solution of BuLi (6.05 mL, 9.68 mmol, 1.6 M solution in hexane) in Et_2O (30 mL) was added dropwise a solution of 2-bromothiazole (806 µl, 8.94 mmol) in the same solvent (30 mL). After the yellow solution had been stirred for 30 min at -78 °C a solution of the ester **1b** (2.5 g, 7.5 mmol) in Et_2O (30 mL) was added slowly. The solution was allowed to warm to -65 °C and was stirred at this temperature for 4 h. after which sat. aq. $NaHCO_3$ was added (30 mL). The mixture was allowed to warm up to room temperature over 20 min and the layers were separated. The aqueous layer was extracted with Et_2O (2 × 30 mL). The combined organic extracts were washed with sat. aq. NaCl (6 mL), dried (Na_2SO_4) and concentrated. Flash chromatography (EtOAc/*n*-heptane, 1/5) to yield the ketone **2b** (2.6 g, 92%) as a yellow oil. To a cold (-78 °C) stirred solution of this ketone (2.6 g, 6.6 mmol) in MeOH was added $NaBH_4$ (506 mg, 14 mmol). The mixture was stirred for 30 min and diluted with acetone (5 mL) and concentrated. The residue was washed with sat. aq. $NaHCO_3$ (3 mL) and extracted with Et_2O (2 × 30 mL), dried (Na_2SO_4) and concentrated. Flash column chromatography (EtOAc/*n*-heptane, 2/3) gave the pure alcohol **2b** (2.6 g, 89%) as a white solid. R_f 0.4 (EtOAc/*n*-heptane, 2/3). $[\alpha]_D^{20}$ -65 (c 0.445; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 2976, 2360, 2340, 1656, 1513, 1247, 1164, 729. ^1H NMR ($CDCl_3$, 400 MHz, ppm) δ: 7.70 (d, 1H, J = 3.2 Hz, Th), 7.32-7.69 (m, 3H, arom), 6.79 (d, 2H, J = 8.5 Hz, arom), 5.50 (m, 1H, allyl), 5.14-4.89 (m, 3H, allyl and CH), 4.35 (d, J = 14.9 Hz, 1H, CH_2), 3.79 (s, 4H, OMe and CH), 3.51 (d, J = 14.9 Hz, 1H, CH_2), 2.78 (m, 1H, CH_2a), 2.36(m, 1H, CH_2b), 1.44 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 159.3, 158.3, 142.9, 134.7, 130.0, 119.0, 118.4, 114.2, 82.0, 74.7, 65.4, 55.9, 55.0, 36.1, 32.6, 29.7, 23.44, 14.89. MS (CI) *m/z*: 405 (M+H). Elemental analysis: calculated for ($C_{21}H_{28}O_4N_2S$) (405.535) C 62.35, H 6.98, N 6.92, found C 61.67, H 7.12, N 6.63.



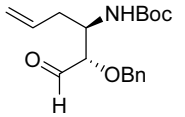
(1*R*,2*S*)-2-[N-(*tert*-Butoxycarbonyl)-*N*-(4-methoxybenzyl)amino]-1-hydroxy-3-allyl-1-(1,3-thiazol-2-yl)propylbenzyl (4)

BnBr (4.3 mL, 36 mmol) and compound **2b** (7.5 g, 18 mmol) were dissolved in DMF and cooled to 0 °C. Sodium hydride (741 mg, 18 mmol) was then added to the reaction mixture. After stirring for half an hour the reaction mixture was quenched with saturated NH_4Cl and extracted with 2-propanol/EtOAc (10/1) and washed with H_2O (4 times) and brine and dried (Na_2SO_4). Flash column chromatography (EtOAc/*n*-heptane, 1/5) gave **4** (8.1 g, 90%) as a colorless oil. R_f 0.2 (EtOAc/*n*-heptane, 1/5). $[\alpha]_D^{20}$ -33 (c 1.60; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 2974, 1688, 1512, 1245, 1165, 735. ^1H NMR ($CDCl_3$, 400 MHz, ppm) temp 55 °C: δ 7.75 (d, J = 2.4, 1H), 7.43-7.24 (m, 8H, arom), 6.75 (m, 2H, PMB), 5.64-5.23 (m, 1H, allyl), 5.12-4.03 (m, 8H, 2CH and allyl, 2CH_2), 3.75 (s, 3H, OMe), 2.40 (m, 1H, CH_2), 2.11 (m, 1H, CH_2), 1.37 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 142.9, 134.9, 128.6, 128.5, 20.22, 117.5, 133.8, 72.6, 55.8, 34.5, 29.1. HRMS (CI) *m/z* calcd for $C_{28}H_{34}O_4N_2S$ $(M)^+$: 494.2239, found: 494.2243. HPLC (OD-H, 1.0 mL/min, *i*-PrOH/hexane, 1/99, 1 µL): retention times (min): 26.162 (ee > 98%).



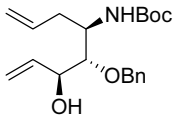
(1*R*,2*S*)-2-[*N*-(*tert*-Butoxycarbonyl)-amino]-1-hydroxy-3-allyl-1-(1,3-thiazol-2-yl) propyl Benzyl (7)

To a solution of **4** (2.46 g, 4.98 mmol) in 50 mL of MeCN/H_2O (4/1) at rt was added $NaHCO_3$ (4.53 g, 14.9 mmol) and ceric ammonium nitrate (CAN; 29.6 g, 14.9 mmol). The reaction was stirred vigorously for 30 min, neutralized with Et_3N (a few drops), and concentrated to dryness. The residue was extracted with EtOAc and washed with H_2O, dried (Na_2SO_4) and concentrated. Flash column chromatography gave (1.23 g, 67%). R_f 0.2 (EtOAc/*n*-heptane, 1/5). $[\alpha]_D^{20}$ -5.3 (c 0.68; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 3448, 1711, 1496, 1365. ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.75 (d, *J* = 2.4 Hz, 1H), 7.43-7.24 (m, 6H), 5.61-5.22 (m, 1H), 5.22-4.45 (m, 3H), 4.67 (d, *J* = 14.8 Hz, 1H), 4.47 (d, *J* = 14.8 Hz, 1H, CH), 4.09 (m, 1H, CH), 2.48-2.18(m, 2H, CH_2), 1,38 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 170.1, 155.5, 142.6, 1347.3, 134.6, 128.4, 119.7, 117.9, 79.4, 79.0, 72.3, 53.8, 36.9, 28.5. HRMS (CI) *m/z* calcd for $C_{20}H_{26}O_3N_2S$ $(M)^+$: 374.1664, found: 374.1664.



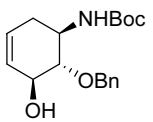
(2*R*,3*S*)-3-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[(benzyl)oxy]-4-allylbutanal (8)

A mixture of compound **7** (1.00 g, 2.67 mmol), activated 4 Å powdered molecular sieves and anhydrous MeCN (26 mL) was stirred at rt for 10 min after which methyl trifluoromethanesulfonate (497 µL, 3.47 mmol) was added. The suspension was stirred for 15 min and concentrated to dryness. The residue was dissolved in MeOH, cooled to 0 °C, and treated with $NaBH_4$ (222 mg, 5.94 mmol). The mixture was stirred at rt for 20 min, diluted with acetone, filtered through Celite, and concentrated. The residue was dissolved in H_2O/CH_3CN (1/10), CuO (1.96 g, 21.3 mmol) and $CuCl_2.H_2O$ (358 mg, 4.54 mmol) were added. The mixture was stirred for 15 min and then filtered through celite. MeCN and most of the water were evaporated (bath temperature not exceeding above 40 °C) to give a brown syrup. The residue was triturated with Et_2O and the liquid face was pipetted through a pad of Florisil (100-200 mesh) to afford a colorless solution (708 mg, 83%). R_f 0.5 (EtOAc/*n*-heptane, 3/2). IR υ_{max} film: (cm^{-1}) 3444, 2975, 1708, 1498, 1365, 1250, 1167, 1053. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 9.64 (s, 1H), 7.42-723 (m, 5H), 5.81-5.61 (m, 1H), 5.09-4.96 (m, 2H), 4.99(br d, *J* = 1.6 Hz, 1H), 4.79 (d, *J* = 11.4 Hz, 1H), 4.47 (d, *J* = 11.4 Hz, 1H), 4.21-4.4.11 (m, 1H), 3.92 (s, 1H), 2.34 (t, *J* = 14.4 Hz, 2H), 1.39 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 155.3, 17.1, 134.0, 128.5, 118.6, 83.4, 79.8, 73.1, 65.9, 50.5, 36.7, 28.6, 15.4.



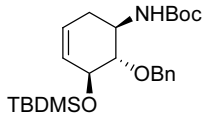
(3*S*,4*S*,5*R*)-5-*(tert*-Butyloxycarbonyl)amino-4-(benzyloxy)-1,7-octadiene-ol (9a)

To a cold solution -78 °C of compound **8** (0.815 g, 2.55 mmol) in THF was added vinlylmagnesium bromide (10.2 mL, 10.2 mmol). The solution was stirred for 4 h and quenched with NH_4Cl. The solvent was evaporated and extracted with Et_2O and water, dried Na_2SO_4, the solvents were evaporated to yield compound **9a** as a colorless liquid (0.963 g, 56%). R_f 0.5 (EtOAc/*n*-heptane, 2/3). $[\alpha]_D^{20}$ +27.3 (c 1.89; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 2976, 1707, 1502, 1171.^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.48-7.25 (m, 5H, arom), 6.19-6.40 (m, 1H, =CH-), 5.82-5.62 (m, 1H, =CH-), 5.72 (d, 1H, *J* = 10.5 Hz, $=CH_2$), 5.12 (d, 1H, *J* = 17.3 Hz, $=CH_2$), 5.11-5.02 (m, 2H, $=CH_2$), 4.77 (d, 2H, *J* = 11.3 Hz, CH_2), 4.66 (d, 2H, *J* = 11.0 Hz, CH_2), 4.80 (br s, 1H, CH), 4.23-4.15 (m, 1H, CH), 4.09-3.80 (m, 1H, CH), 2.31 (br t, 2H, *J* = 7.2 Hz, CH_2), 1.41 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 135.0, 128.9, 128.8, 118.1, 118.0, 79.9, 75.6, 73.7, 51.0, 38.5, 29.1. HRMS (CI) *m/z* calcd for $C_{24}H_{30}O_4N$ $(M)^+$: 348.2174, found: 348.2167.



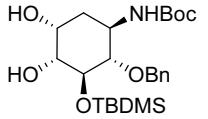
(1*R*,5*S*,6*S*)-6-benzyloxy-5-hydroxy-cyclohex-3-enyl-1-amino(*tert*-butyloxycarbonyl) (10)

To a mixture of compound **9a** (20 mg, 5.7 mmol**)** in CH_2Cl_2 was added the mono-substituted imidazolylidene ligand (3 mg, 5 mol%) The mixture was stirred for 2 h at rt and evaporated dried (Na_2SO_4). Column chromatography (EtOAc/*n*-heptane, 1/5) gave **10** (18 mg, 98%). as a white solid. R_f 0.4 (EtOAc/*n*-heptane, 1/1). $[\alpha]_D^{20}$ +20.0 (c 0.57; CH_2Cl_2). Mp: 126 °C. IR υ_{max} film: cm^{-1} 3342, 1681, 1531, 1168, 734. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.43-7.19 (m, 5H, arom), 5.79-5.40 (m, 2H, CH=CH), 4.92-4.68 (m, 3H, Bn and CH), 4.32 (br s, 1H, NH), 4.05-3.78 (m, 1H, CH), 3.47 (q, *J* = 6.0 Hz, 1H, CH), 2.68-2.42 (m, 1H, CH_2), 2.20-1.95 (m, 2H, CH_2 and OH), 1.44 (s, 1H, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 155.9, 138.6, 128.8, 128.1, 126.9, 82.0, 73.4, 71.5, 31.6, 28.6. HRMS (CI) *m/z* calcd for $C_{18}H_{26}NO_4$ $(M+H)^+$: 320.1861, found: 320.1860.



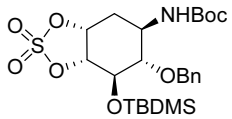
(1*R*,5*S*,6*S*)-6-benzyloxy-5-(*tert*-butyldimethylsilyl)oxy-3-cyclohexenyl-1-(*tert*-butyloxycarbonyl)amine (11)

Compound **10** (167 mg, 0.523 mmol) was dissolved in CH_2Cl_2 (5 mL) and cooled to -20 °C then *tert*-butyldimethylsilyl trifluoromethanesulfonate (156 µl, 0.676 mmol) and DIPEA (127 µl, 0.732 mmol) were added to the reaction mixture. After stirring for 30 min the mixture was quenched with sat. aq. $NaHCO_3$ and dried with Na_2SO_4, evaporated and purified by flash column chromatography (EtOAc/*n*-heptane, 1/10) gave (186 mg, 84%) of compound **11** as a white solid. R_f 0.68 (EtOAc/*n*-heptane, 2/3). $[\alpha]_D^{20}$ +12.6 (c 0.27; CH_2Cl_2). mp 78 °C. IR υ_{max} film: (cm^{-1}) 2956, 2885, 1714, 1513, 1114, 840. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.19-39 (m, 5H, Bn), 5.69-5.82 (m, 1H, H_5), 5.49-5.68 (m, 1H, H_4), 4.62 (dd, J = 12.0 Hz, 2H, CH_2Bn), 4.18 (m, 2H, H_3 and H_1), 3.48 (dd, J = 3.5 Hz, 1H, H_2), 2.34-2.53 (m, 1H, H_6), 2.18-1.99 (m, 1H, H_6), 1.37 (s, 9H, *t*-Bu), 0.86 (s, 9H, *t*-Bu), 0.03 (s, 6H, 2Me). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm); δ 160.5, 143.3, 133.5, 132.8, 131.9, 83.9, 83.2, 77.4, 73.6, 36.9, 33.8, 30.8, 22.9, 0.4, 0.0. HRMS (CI) *m/z* calcd for $C_{24}H_{40}O_4NSi\,(M+H)^+$: 434.2727, found: 434.2781.



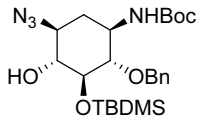
(1*R*,2*R*,3*S*,4*S*,5*R*)-5-(*tert*-Butyloxycarbonyl)amino-4-benzyloxy-3-[(*tert*-butyldimethylsilyl)oxy]cyclohexane-1,2-diol (14)

To a vigorously stirred solution of the alkene **11** (15 mg, 0.046 mmol) in EtOAc and CH_3CN (200 µl, 1:1) at 0 °C (ice bath) was added a solution of $RuCl_3{\cdot}H_2O$ (cat) and $NaIO_4$ (15 mg, 0.069 mmol) in distilled H_2O. The two fase system was stirred vigorously for 3 min and quenched with sat. aq. solution of Na_2SO_3. The aqueous face was separated and extracted with EtOAc. The combined organic abstracts were dried (Na_2SO_4) and concentrated. Flash column chromatography (EtOAC/*n*-heptane, 1/5) gave (15 mg, 70%) of the diol (**14**) as colorless oil. R_f 0.1 (EtOAc/*n*-heptane, 2/3). $[\alpha]_D^{20}$ +12.8 (c 0.445; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 2925, 2358, 1778, 1697, 1508, 1135, 1081, 838. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.36-7.12 (m, 5H), 4.71 (d, J = 11.6 Hz, 2H), 4.53 (d, J = 11.6 Hz, 2H), 4.21 (m, 1H, CH), 4.1 (m, 1H, CH), 3.97 (m, 1H), 3.75 (m, 1H), 3.44 (m, 1H), 3.27 (br d, J = 9.8 Hz, 1H), 2.02-1.89 (m, 2H), 1.42 (s, 9H, *t*-Bu), 0.88 (s, 9H, *t*-Bu), 0.03 (s, 6H, $SiMe_2$). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 154.8, 136.7, 128.3, 79.4, 73.7, 72.8, 72.3, 64.7, 60.6, 48.1, 30.1, 28.7, 26.0, 18.1, 14.6, -4.7. HRMS (CI) *m/z* calcd for $C_{24}H_{42}O_6NSi\,(M+H)^+$: 468.2781, found: 468.2785.



(1*R*,2*S*,3*R*,4*S*,5*R*)-5-(*tert*-Butyloxycarbonyl)amino-4-benzyloxy-3-[(*tert*-butyldimethylsilyl)oxy]cyclohexane-1,2-sulfate (15)

To a solution of diol **14** (23 mg, 0.024 mmol) and thionyl chloride (1.8 µL, 0.025 mmol) in EtOAc was added a solution of pyridine (4.1 µL, 0.051 mmol). The mixture was stirred and was not allowed to rise above rt. When TLC analysis showed complete formation of the cyclic sulfate the mixture was diluted with EtOAc and extracted with H_2O. The organic phase was dried (Na_2SO_4) and concentrated. The residue was dissolved in a mixture of CH_2Cl_2, MeCN and water (2:2:3), $NaIO_4$ (10 mg, 0.048 mmol) and $RuCl_3{\cdot}H_2O$ (catalytic) were added and the mixture was stirred for 1h at 20 °C. The mixture was filtered and the filtrate was diluted with CH_2Cl_2, the organic layer was extracted with water, dried ($MgSO_4$) and concentrated. Flash column chromatography (EtOAc/*n*-heptane, 1/5) gave compound **15** as a white solid (20 mg, 80%). R_f 0.5 (EtOAc/*n*-heptane, 2/3). IR υ_{max} film: (cm^{-1}) 2923, 1687, 1328, 1212, 840. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.48-7.25 (m, 5H, Bn), 5.18-5.12 (m, 1H, CH), 4.70 (dd, 2H, J = 11.7 Hz, CH_2), 4.71-4.70 (m, 1H, CH), 4.25-4.18 (m, 1H, CH), 3.74-68 (m,1H, CH), 3.52-3.33 (m, 1H, CH), 2.63 (dt, 1H, CH_2), 1.40 (s, 9H, *t*-Bu), 1.48-1.40 (m, 1H, CH_2), 0.91 (s, 9H, *t*-Bu), 0.15 (s, 3H, SiMe), 0.08 (s, 3H, SiMe).



(1*R*,2*S*,3*S*,4*R*,6*S*)-6-azido-4-(*tert*-butyloxycarbonyl)amino-3-benzyloxy-2-[(*tert*-butyldimethylsilyl)oxy]cyclohexane-1-ol (16)

Compound **15** (10 mg, 0.019 mmol) was dissolved in DMF (500 µL). The reaction mixture was heated to 80 °C and LiN_3 (2.4 mg, 0.046 mmol) was added to the reaction mixture. The reaction mixture was heated until TLC analysis showed conversion of the cyclic sulfate into baseline material. After stirring for 2 h, the reaction mixture was evaporated and dissolved in THF. A drop of water and H_2SO_4 (5 µL) were added and the reaction mixture was quenched with $NaHCO_3$ and extracted with EtOAc, dried Na_2SO_4 and purified by flash column chromatography (EtOAc/*n*-heptane, 1/5) to yield the diol **16** (6 mg, 60%) as a white solid. R_f 0.3 (EtOAc/*n*-heptane, 2/3). $[\alpha]_D^{20}$ – 21.0 (c 0.10; CH_2Cl_2). IR υ_{max}

film: (cm^{-1}) 2928, 2103, 1689, 1169, 1069, 610. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.43-7.24 (m, 5H, arom), 4.50 (dd, J = 7.8, 11.4 Hz, 2H, CH_2), 3.72-3.44 (m, 4H, CH), 3.22-3.11 (m, 1H, CH), 2.62-47 (m, 1H, CH_2), 2.32-2.26 (m, 1H, CH_2), 1.40 (s, 9H, *t*-Bu), 0.93 (s, 9H, *t*-Bu), 0.32 (s, 6H, $SiMe_2$). HRMS (CI) *m/z* calcd for $C_{24}H_{41}O_5N_4Si\,(M)^+$: 493.2846, found: 493.2847.

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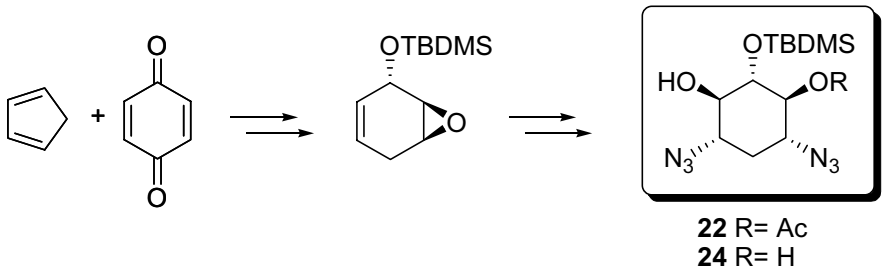
3 Preparation of a 1,3-diazidocyclitol as a versatile 2-deoxystreptamine precursor[1]

Abstract

Synthesis of a direct 2-deoxystreptamine (2-DOS) precursor, a common structure in the majority of clinically important aminoglycosides, is presented. The synthetic route described in this chapter starts from *p*-benzoquinone and cyclopentadiene and comprises a Pd(0)-catalyzed rearrangement and a retro-Diels–Alder reaction by flash vacuum thermolysis. Final stereoselective introduction of the desired *trans*-azidoalcohol was explored with a variety of methods *e.g.* via an Yb(III)-directed regioselective epoxide opening, via diepoxide opening, or via dihydroxylation, cyclic sulfate forming and opening with lithium azide. The obtained orthogonally protected diazidocyclohexitol (DACH) derivatives **22** and **24** are suitable 2-DOS precursors, conveniently protected for incorporation in new aminoglycoside entities.



22 R= Ac
24 R= H

[1] Part of this chapter has been published in: G. F. Busscher, S. Groothuys, R. de Gelder, F. P. J. T. Rutjes, F. L. van Delft *J. Org. Chem.* **2004**, *96*, 4477-4481.

3.1 Introduction

Since the discovery of the aminoglycoside antibiotic streptomycin in 1944,[1] the family of aminoglycosides has steadily grown into a powerful class of antibiotics with a broad antibacterial spectrum and proven efficacy particularly against aerobic Gram-negative bacteria. Nevertheless, extensive clinical use of the aminoglycosides is limited, mostly due to the associated nephro- and ototoxicities.[2] Another disadvantage is the global development of microbial resistance as the result of structural modification by bacterial enzymes; aminoglycoside phosphotransferases (APH), adenyltransferases (AAD or ANT) and acetyltransferases (AAC).[3,4] These circumstances validate research into novel aminoglycoside analogues which do not display the undesirable features but maintain a strong bactericidal effect. This notion has already awakened the chemical community and the number of papers along this line is rapidly increasing.[5] Surprisingly, however, none of these reports describes an efficient way to prepare the core diaminocyclohexanetriol 2-deoxystreptamine (2-DOS, Figure 1) common to (nearly) all of the known aminoglycoside antibiotics.[5]

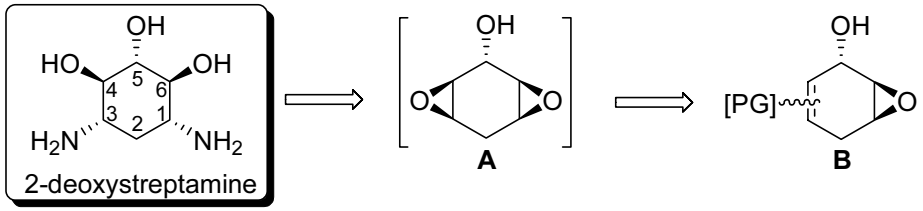


Figure 1. Retrosynthetic analysis of 2-DOS.

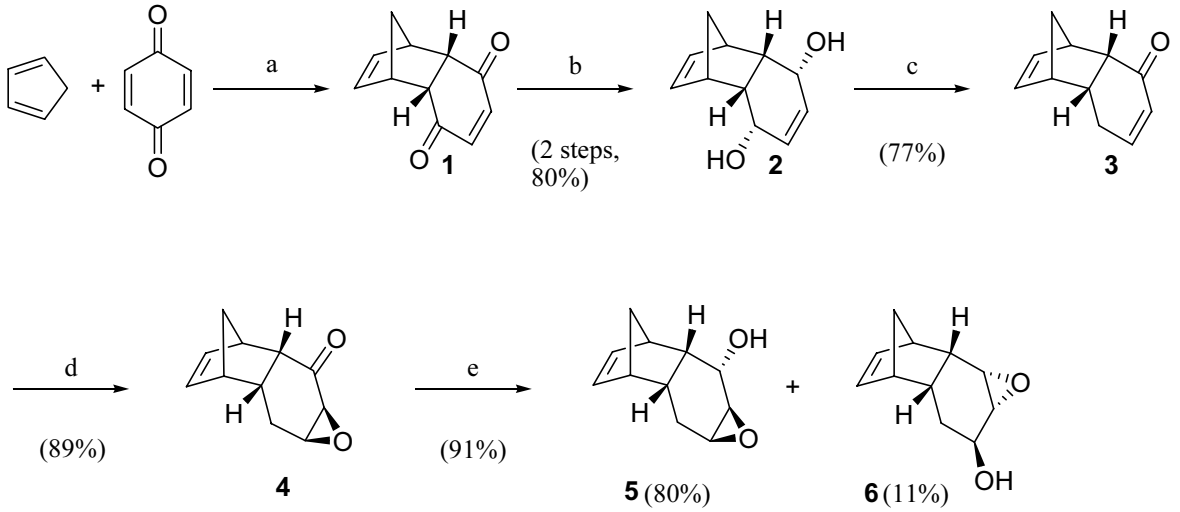
Synthetic routes toward 2-DOS that have appeared in literature require numerous synthetic steps and offer minimal flexibility in protective groups[6,7,8] or require expensive starting material (see Chapter 1). As a result, to date the most practical method to synthesize 2-DOS is by degradation of natural neomycin.[9,10,11] However, the initially obtained "naked" *meso* compound still demands desymmetrization as well as extensive protective group manipulations before successful incorporation in aminoglycoside entities can be ensured. Based on this reasoning we set out to investigate a practical synthetic route toward a 2-DOS precursor that is suitable to serve as scaffold for either 4,5- or 4,6-linked aminoglycoside antibiotics.

3.2 Unexpected cage structures *en route* to 2-DOS

As becomes clear from Figure 1, the target molecule 2-DOS shows a remarkable structural simplicity particularly due to the internal plane of symmetry. An obvious retrosynthetic approach therefore suggests introduction of both the amino functionalities by dual nucleophilic opening of diepoxide **A**. This diepoxide has been described in literature, but it is unstable and the synthetic

route leading to it is by no means straightforward.[6,12] Unfortunately, obtention of **A** by epoxidation of cyclohexane-2,5-dien-1-ol (not drawn) seemed not feasible to us since the inherent instability of the latter molecule precludes its use in chemical synthesis. In chapter 2 we described the synthesis of an enantiopure and fully orthogonally protected 2-DOS by using a stepwise approach starting from D-allylglycine.[13] The latter route provides a highly useful 2-DOS scaffold for the preparation of new aminoglycoside antibiotics but in this chapter we reasoned a shorter route toward a versatile 2-DOS precursor would be valuable.

Scheme 1.



Reagents and conditions: (a) MeOH, 0-5 °C, 2.5 h; (b) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, 0-5 °C, 1 h; (c) $PdCl_2$(dppf), HCO_2NH_4, MeCN, Δ, 45 min; (d) H_2O_2, 0.2 M NaOH, CH_2Cl_2/MeOH, 1/1, rt, 30 min; (e) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, -78 °C, 30 min.

Our synthesis starts from the readily accessible Diels–Alder condensation product **1** of cyclopentadiene and *p*-benzoquinone (Scheme 1). Reduction under Luche conditions according to a known protocol[14,15] led to diol **2** which was converted into the enone **3** via a 1,4-hydrogen migration catalyzed by *in situ* formed Pd(0), according to Takano *et al.*[16,17] In our hands, however, it was not possible to reproduce the reported yield under the described conditions ($PdCl_2(PPh_3)_2$ in MeCN), since an appreciable amount (16%) of starting material remained (Table 1, entry 1). We therefore further investigated the optimal reaction conditions for this transformation, the parallel syntheses were performed with a synthesis robot (Figure 2). In DMF we saw minor amounts of **3** or no product formed (entry 5, 6), which also applied to the use of other sources of palladium in MeCN (entry 2, 4). On the contrary, with $PdCl_2$(dppf) as catalyst we found only minor residual starting material and a much improved yield of **3** (entry 3).

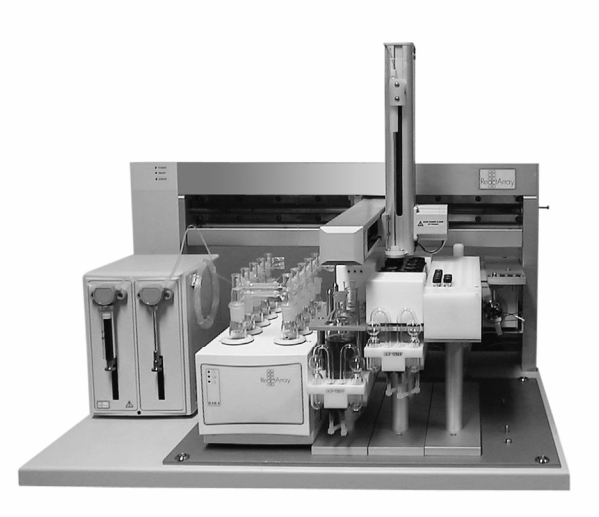


Figure 2. System used for optimization of the palladium catalyzed reaction.

Table 1. Optimization of the palladium(0)-catalyzed 1,4-hydrogen migration [a]

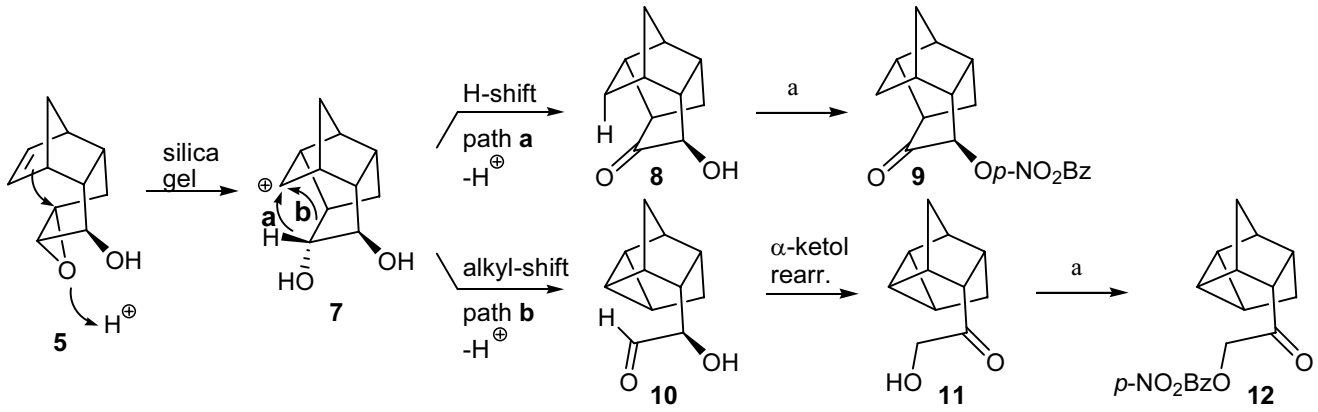
	solvent	catalyst	**2** yield (%)[b]	**3** yield (%)[b]
1	MeCN	$PdCl_2(PPh_3)_2$	16	71 (49)
2	MeCN	$PdCl_2(MeCN)_2$	-[c]	-[c]
3	MeCN	$PdCl_2$(dppf)	9	89 (77)
4	MeCN	$Pd(OAc)_2$(dppe)	100	-
5	DMF	$PdCl_2(PPh_3)_2$	100	-
6	DMF	$PdCl_2$(dppf)	69	29

[a] standard procedure is used, with given catalyst, and solvent. [b] GC yield (isolated yield).[c] decomposition.

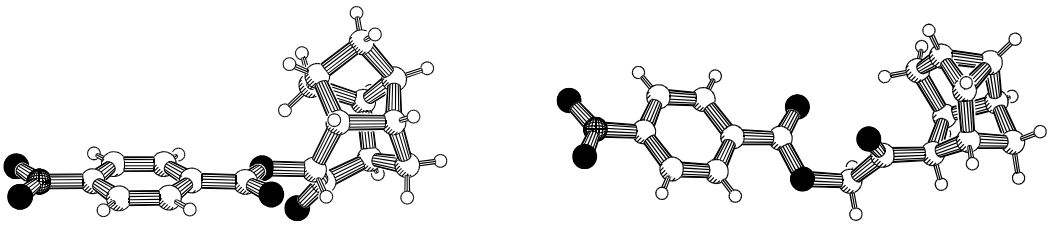
Having established the palladium(0)-catalyzed hydrogen migration, the next step is a nucleophilic epoxidation reaction. Thus, reacting α,β-unsaturated ketone **3** with hydrogen peroxide under basic conditions afforded epoxide **4** in a yield of 89% (Scheme 1). The following step, reduction of the ketone to alcohol **5** proved troublesome and was inevitably accompanied by varying amounts of the Payne-rearranged product (**6**). Optimal conditions to keep this side-reaction to a minimum involved reduction under Luche conditions[15] at -78 °C to afford alcohol **5** and **6** in a favorable 7:1 ratio of isomers, which could be easily separated by column chromatography. To investigate the energy difference AM1 molecular mechanics were performed. The molecular mechanics, using MOPAC/AM1, and quantum mechanics using B3LYP 6-31G*, show that there is little energy difference between Payne-rearranged product **6** and starting compound **5**, predicting $\Delta E = 1.8$ and 0.75 kcal/mole, respectively. In order to define whether the epoxides **5** and **6** are in a dynamic equilibrium under the basic conditions applied, each epoxy alcohol was individually treated with sodium hydride. It turned out that only the *transoid*-epoxy alcohol (**5**) (partially) rearranged to the regioisomeric 1,2-*cisoid*-epoxy alcohol **6**, whereas the reverse reaction did not take place. The results indicate that, although it stops at a certain ratio of inverted and non inverted product, the Payne rearrangement is not in equilibrium. The results found here are in agreement with the reported (un)stability of an α,β-epoxy-cyclopentanol instead of the above mentioned α,β-epoxy-cyclohexanol.[18] The reason for the rearrangement not to proceed beyond a certain point remains unclear at this moment.

Although we were satisfied with the fact that Payne rearrangement could be kept to a minimum under an appropriate protocol, we discovered that the conversion of **4** to **5** requires additional caution, due to the high acid-sensitivity of the desired product **5**,[19] purification on silica gel led to the formation of two new products. The structure of the latter inseparable compounds could not be fully established but since spectroscopic analysis revealed the presence of an alcohol, the unknown product was converted into its *p*-nitrobenzoate derivative, since *p*-nitrobenzoates are known to crystallize easily (Scheme 2). Subsequent separation by selective crystallization of the *p*-nitrobenzoate derivatives, followed by X-ray analysis revealed that the rather striking tetracyclic structures **9** and **12** had been formed (Figure 3).[20]

Scheme 2.



Reagents and conditions: (a) *p*-NO_2-BzCl, Et_3N, DMAP, CH_2Cl_2.



9 **12**

Figure 3. Platon visualization[25] of the X-ray structures of **9** and **12**.

We suggest that the mechanism leading to **9** and **12** involves acid activation (silica gel) of the epoxide of **5** which has the double bond π-electrons ideally positioned for nucleophilic attack. The resulting intermediate carbocation **7** undergoes one of two possible rearrangements, involving either an H-shift (path **a**) or alkyl-shift (path **b**). Although the α-hydroxy aldehyde **10** as such could not be identified, it seems likely that formation of the nitrobenzoate **12** can be explained via α-ketol rearrangement of **10** to the more stable ketone **11**. In addition, when epoxyalcohol **5** was stirred in

CH_2Cl_2 in the presence of silica gel the same rearrangement takes place. To prove that the free hydroxyl has no influence in the predicted mechanism we protected the free hydroxyl of compound **5** with an acetyl group. Stirring of the latter in CH_2Cl_2 with silica gel for 48 hours followed by removal of the acetyl group with potassium cyanide in MeOH gave again the cage structures **8** and **11**. Gratifyingly, rearrangement could be completely suppressed by pre-eluting the column with 1% Et_3N to give **5** in a final isolated yield of 80% (Scheme 1).

3.3 Retro-Diels–Alder reaction by flash vacuum thermolysis and epoxide opening

Having the tricyclic structure **5** in hand, the desired retro-Diels–Alder reaction could be investigated. Such reactions, typically executed in high-boiling ethereal solvents, are often accompanied by side-products and the solvent is inherently difficult to remove. These disadvantages can be elegantly circumvented by application of flash vacuum thermolysis (FVT, Figure 4).[21] The first attempt to afford epoxyalcohol **13** yielded only 22% of the desired product and 75% starting material (Table 2, entry 1). In the next attempt a raise in thermolysis temperature (500→550 °C) gave a yield of 62% and only 3% of the starting material remained. However this reaction furnished beside these two products a reasonable amount of a side product, the identity of which we have not been able to establish (entry 2).

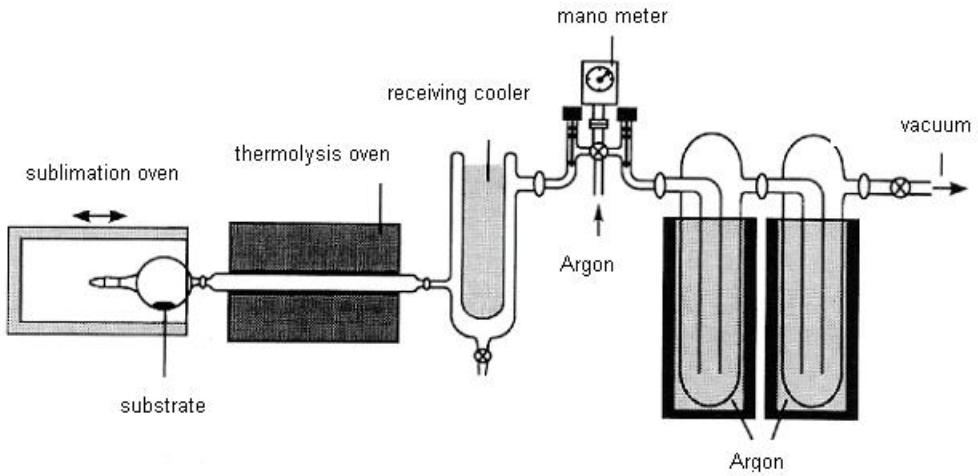
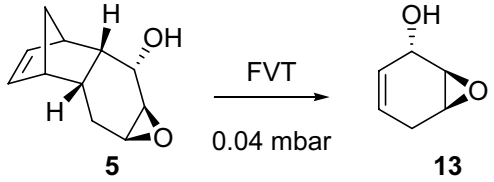


Figure 4. Flash vacuum thermolysis set-up.

After a few more optimization reactions (entry 3, 4, 5) it was found that the best result was obtained with sublimation at 80 °C, thermolysis at 600 °C and the pressure around 0.04 mbar to afford compound **13** cleanly in 84% yield (entry 6).

Table 2. Optimization of the flash vacuum thermolysis reaction[a]



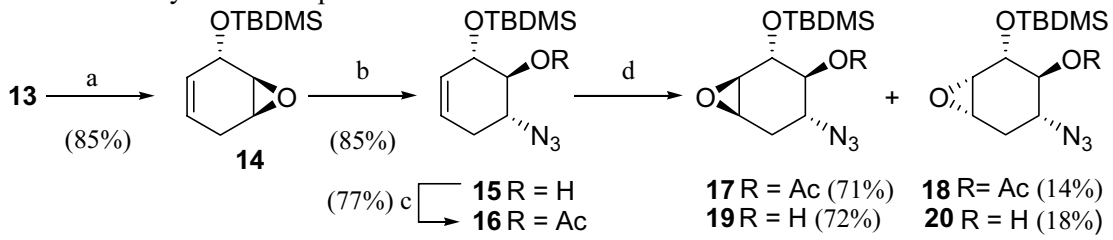
5 **13**

	sublimation (°C)	thermolysis (°C)	yield (**5**) (%)[a]	yield (**13**) (%)[a]	UFO[b] (%)
1	120	500	75	22	3
2	120	550	3	62	35
3	100	500	39	30	31
4	80	500	45	45	10
5	80	550	13	81	5
6	80	600	-	96 (84)	4

[a] GC yield (isolated yield)[b] Unidentified flying object

In the following steps the hydroxyl was protected with a *tert*-butyldimethylsilyl group and the epoxide was converted to azido alcohol **15** (Scheme 3). The free hydroxyl was now acetylated and the remaining double bond was epoxidized, via *in situ* dioxirane formation reported by Yang *et al,*[22] leading to predominantly the *trans*-epoxide **17** (71%) and a minor amount of the *cis*-isomer **18** (ratio 4:1). The cyclohexene ring bearing a free hydroxyl was also epoxidized, in this case with *m*-CPBA. Giving the *trans* and *cis* epoxide (**19** and **20**) in a yield of 72% and 18%, respectively. Epoxidation could also be performed with the *in situ* formed dioxirane but although this reaction proceeds faster, it neither gave a better ratio between *cis* and *trans* epoxide, nor a better yield.

Scheme 3. Synthesis of epoxides **17**-**20**

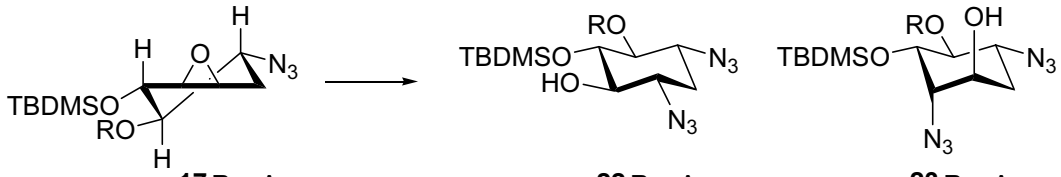


Reagents and conditions: (a) TBDMSCl, DIPEA, DMAP, CH_2Cl_2, rt, 5 h; (b) NaN_3, NH_4Cl, MeOH, Δ, 40 h; (c)Ac_2O, DMAP, CH_2Cl_2,rt, 1h; (d) Oxone®, chlorocyclohexanone, $NaHCO_3$, MeCN/H_2O 3:2, 1 h; (d) or *m*-CPBA, CH_2Cl_2, rt, 24 h, rt.

The final step, involving another regioselective epoxide opening of epoxide 1**7** or **19**, proved to be more troublesome (Table 3). Our first attempt to open epoxide **17** with NaN_3 in MeOH predominantly led to the formation of azido alcohol **23** and only to a lesser extent to the preferred regioisomer **22** (entry 1), presumably due to a favored *trans*-diaxial opening of the epoxide as dictated by the Fürst-Plattner rule.[23] Opening with sodium azide and lithium perchlorate did not improve matters since after prolonged stirring mainly starting material was recovered (48h)

(entry 2). After some unsuccessful variation of reaction conditions, a paper by Delgado *et al*. stimulated us to investigate chelation-controlled $Yb(OTf)_3$-catalyzed azidolysis of epoxides (Figure 5).[24] Much to our satisfaction, under the suggested conditions with sodium azide, ytterbium(III) triflate and triethylamine, the 1,3-diazidocyclitol (1,3-DACH) **24** was formed as the only regioisomer (Table 3, entry 5), most likely from nucleophilic attack at the all-axial conformer, formed by chelation of ytterbium(III) with both the free (deprotonated) hydroxyl and the epoxide (Figure 5). The likeliness of such a mechanism was substantiated when we observed that application of the $Yb(OTf)_3$-catalyzed procedure to the protected derivative **17** did not lead to any reaction (entry 3).

Table 3. Optimization of the epoxide opening of **17** and **19**



17 R = Ac
19 R = H
21 R = TMS

22 R = Ac
24 R = H

23 R = Ac
25 R = H

	s.m. [a]	conditions	solvent	**22**:**23** [b]	isolated yield **22** (%)
1	**17**	NaN_3, NH_4Cl, 80 °C	MeOH	9:32	21
2	**17**	NaN_3, $LiClO_4$ 80 °C	toluene	-	-[c]
3	**17**	NaN_3, $Yb(OTf)_3$, Et_3N, 60 °C	toluene	-	-[c]
		conditions	solvent	**24**:**25** [b]	yield **24** (%)
4	**19**	NaN_3, NH_4Cl, 80 °C	MeOH	1:2	10
5	**19**	NaN_3, $Yb(OTf)_3$, Et_3N, 80 °C	toluene	>95:5	49
6	**19**	NaN_3, $Yb(OTf)_3$, 60 °C	MeOH	-	-
7	**19**	$TMSN_3$, $Yb(OTf)_3$, Et_3N, 60 °C	toluene	-	-[d]
8	**19**	$(Me_2N)_2C{=}NH_2N_3$, $Yb(OTf)_3$, Et_3N, 60 °C	toluene		18
9	**19**	NaN_3, $Yb(OTf)_3$, Et_3N, 280 W, 135 °C	toluene	>95:5	70[e]
10	**19**	NaN_3, $Yb(OTf)_3$, Et_3N, 80 °C, 4Å MS	toluene	>95:5	82[e]

[a] s.m. = starting material [b] regioisomeric ratio determined by ^1H NMR [c] mainly starting material **17** [d] major compound **21** (in 70% yield) [e] BORSM = based on recovered starting material.

Unfortunately, conversion was never complete, since after 4 days of refluxing substantial amounts of starting material remained and only 49% of the product could be isolated. Replacement of the sodium azide by $TMSN_3$ was not a fortunate choice since the silylated product **21** was obtained (entry 7). Replacement of the sodium azide with tetramethylguanidinium azide yielded the product

in a disappointing yield of 18% accompanied by several side products (entry 9). Replacement of toluene by MeOH was again not fortuitous, but by carrying out the reaction in a microwave at 280 W and at 135 °C (entry 9), product **24** was obtained in a yield of 70%. Finally, the optimal result was achieved by addition of molecular sieves to the reaction mixture, to give a yield of 82%. The structural identity of **24** was unequivocally established by X-ray analysis (Figure 6).[20]

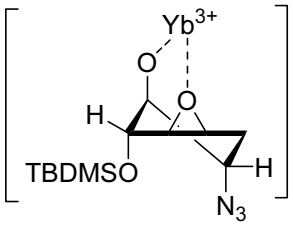


Figure 5.

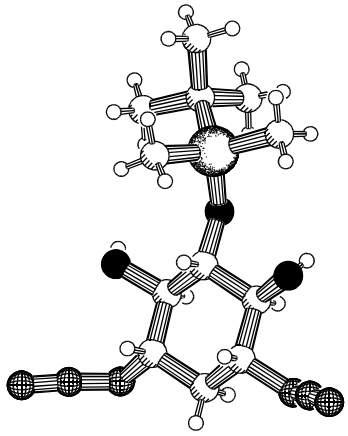
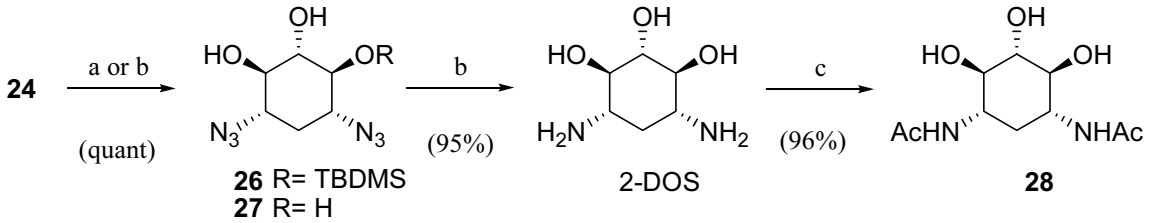


Figure 6. Platon visualization [25] of the X-ray structure of **24**.

The convenience of the diazido derivative **24** above carbamate-protected 2-DOS is reflected in its excellent solubility in organic solvents and straightforward spectral analysis. Finally, the applicability of **24** as a versatile scaffold for the preparation of new 4,6-linked aminoglycoside type compounds[6e,7] was established by its smooth conversion into 2-DOS. To this end, the TBDMS protective group was removed with 1N HCl in methanol, since attempted desilylation with TBAF surprisingly resulted in formation of the TBDMS migration to a neighboring hydroxyl (**26**). The next step was hydrogenation which yielded 2-DOS in 95% yield for the two steps (Scheme 4). Comparison of spectral data of the diacetate derivative **28** with reported data[26] provided further evidence of the structural identity of **24**.

Scheme 4. Conversion of **24** into 2-DOS

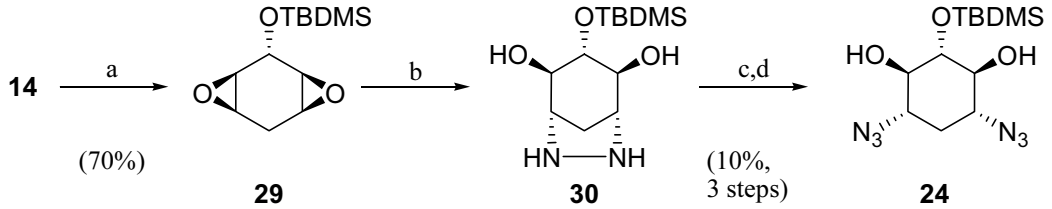


Reagents and conditions: (a) TBAF, THF, 0 °C, 30 min; (b) 1N HCl, MeOH, rt, 16 h; (b) Pd-C, H_2, MeOH, 16 h; (c) Ac_2O, Et_3N, MeOH/H_2O, 1/1, 3h.

3.4 Synthesis of 2-DOS *via* the diepoxide

A potentially more direct route toward 2-DOS follows direct epoxidation of the double bond of the TBDMS protected FVT product **14**. It was reasoned that the obtained diepoxide could be subsequently ring opened with sodium azide in a single step. Thus, starting from the protected epoxy alcohol **14**, the double bond was epoxidized with *m*-CPBA to obtain the diepoxide **29** in 70% yield (Scheme 5). Although formation of the *trans* diepoxide could not be completely circumvented, the *cis* and *trans* epoxide were formed in a ratio of 9:1 (in favor of the desired *cis*-diastereomer), corroborated by a crystal structure of the major diepoxide.

Scheme 5. Dual epoxide opening toward **24**



Reagents and conditions: (a) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, rt, 48 h; (b) hydrazine, EtOH, Δ, 4 h; (c) Amberlite IRA 400, Ra-Ni, H_2, H_2O/MeOH, 1/1, 16 h; (d) TfN_3, $ZnCl_2$, Et_3N, CH_2Cl_2/MeOH/H_2O, 3/10/3, rt, 3 h.

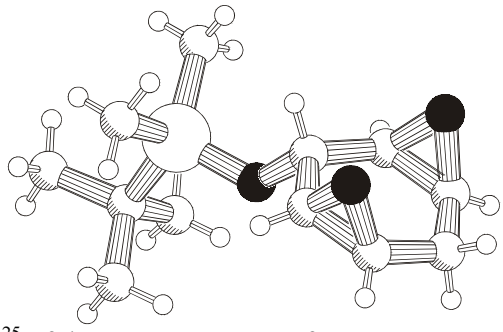


Figure 7. Platon visualization [25] of the X-ray structure of **29**.

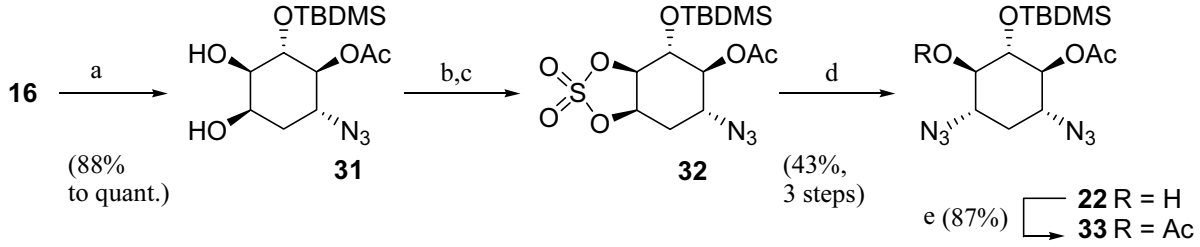
Opening with sodium azide under ammonium chloride catalysis led to the formation of mainly the wrong regioisomers which was not surprising based on our earlier findings. More surprisingly, opening of epoxide **29** with sodium azide in the presence of zinc sulfate gave the 2-DOS derivative in a disappointing yield of 15% and the other regioisomer as the main product in a yield of 54%, opposite to the reported findings of Prinzbach. [27] Little success was also met when the diepoxide was opened with sodium azide under $Yb(OTf)_3$-catalyzed conditions because after prolonged stirring (7 days, 80 °C) only opening of a single epoxide and minor amounts of the diol were observed. A more successful strategy involved opening with hydrazine which has the inherent advantage that the second nucleophilic addition proceeds intramolecularly, and hence with proper regiochemistry. Subsequent reduction of the resulting crude product with Ra-Ni in the presence of basic Amberlite gave the TBDMS protected 2-DOS and two other unidentified side-products. The

last step, diazotransfer catalyzed by $ZnCl_2$, gave the corresponding diazido derivative **24** in a disappointing yield of 10% for three steps.[28] In conclusion, in 10 steps and an overall yield of 2.2% the 1,3-DACH **24** was synthesized.

3.5 Synthesis of orthogonally protected 2-DOS via dihydroxylation and cyclic sulfate formation

Since it was found impossible to drive the epoxide opening of **19** to completion, an approach involving cyclic sulfate methodology, applied successfully earlier in chapter 2, was followed. Thus, the double bond of intermediate **16** was reacted with catalytic osmium tetroxide, but even after prolonged stirring no conversion of starting material was observed,[29] even when a stoichiometric amount of osmiumtetroxide was used. Therefore, cyclohexene **16** was reacted with ruthenium(III) chloride and sodium periodate, leading to the desired diol in a yield of 88% (Scheme 6).[30] However, since these conditions can easily lead to over–oxidation –by oxidative cleavage of the resulting diol– we were hesitant to employ these conditions on a large scale. Therefore, other dihydroxylation methods were explored. For example, it is known that the presence of $CH_3SO_2NH_2$ in the OsO_4 catalyzed dihydroxylation reaction leads to shorter reaction times, occasionally 50-fold catalysis is observed, by accelerating the osmate ester hydrolysis.[31,32] Therefore, alkene **16** was reacted with OsO_4 in the presence of 1 equiv. of sulfonamide, leading to the quantitative isolation of diol **31**. The last transformations to obtain 2-DOS involve reaction of the diol with thionylchloride and oxidation to cyclic sulfate **32**, followed by opening of the corresponding sulfate with lithium azide. Following such a sequence of events yielded the orthogonally protected 2-DOS precursor (**22**) cleanly, but in a rather low 43% yield.[33] To prove the stereochemistry of compound **22**, the free hydroxyl was acetylated to give diacetylated structure **33**, spectral data of which were in full agreement with the spectra obtained by acetylation of compound **24**.

Scheme 6. Conversion of **16** toward orthogonally protected 2-DOS analogue **22**



Reagents and conditions: (a) $RuCl_3$, $NaIO_4$, EtOAc/MeCN/H_2O (3/3/1), 0 °C, 3 min (88%) or OsO_4, $CH_3SO_2NH_2$, *t*-BuOH/H_2O (1/1) (quant.); (b) $SOCl_2$, pyridine, EtOAc, 0 °C, 30 min. (c) $NaIO_4$, $RuCl_3.H_2O$, CH_2Cl_2/MeCN/H_2O (2/2/3), 0 °C, 1 h; (d) LiN_3, wet DMF, Δ, 4 h then H_2SO_4, THF, H_2O, rt, 30 min (43% for 3 steps) (e) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 1h, (87%).

3.6 Concluding remarks

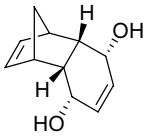
Several synthetic routes of 2-DOS proceeding via a retro-Diels–Alder reaction were developed successfully; DACH **24** was synthesized in 10 steps and an overall yield of 16% (§**3.3**), and via the diepoxide opening of (**29**) in 10 steps and an overall yield of 2.2% (§**3.4**). The orthogonally protected 2-DOS entity **22** was synthesized in 13 steps and an overall yield of 8.8% (§**3.5**). The most straightforward route proceeds via epoxide opening but the last step is not ideal since the reaction fails to go to completion under a variety of conditions. Synthesis of the *di*epoxide and dual opening of the corresponding epoxides has no advantages over the previous route. Similarly, application of cyclic sulfate methodology does not improve the overall yield but opens possibilities for desymmetrization earlier in the synthesis, since the final product is not necessarily *meso*. The obtained diazidocyclitols, which can be obtained at gram-scale following a route presented here, are suitable 2-DOS precursors and moreover conveniently protected for incorporation in new aminoglycoside entities.

Finally, an interesting intramolecular electrophilic cyclisation reaction was discovered serendipitously, leading to unique and highly bridged cage structures.

3.7 Acknowledgements

S. Groothuys and B. Verheijen are gratefully acknowledged for their contribution to this chapter. Dr. A. J. H. Klunder (organic chemistry, Radboud University Nijmegen, The Netherlands) is acknowledged for the helpful discussions and advice on the flash vacuum thermolysis methodology and Dr. H. Borkent (center for molecular and biomolecular Informatics, Radboud University Nijmegen, The Netherlands) for the AM1 calculations and quantum mechanics. Dr. R. de Gelder (inorganic chemistry, Radboud University Nijmegen, The Netherlands) is kindly acknowledged for elucidation of the X-ray structures.

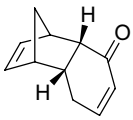
3.8 Experimental Section



(1*R*,2*S*,3*R*,6*S*,7*R*,8*S*)-Tricyclo[6.2.1.0^{2,7}]undeca-4,9-diene-3,6-diol (2)

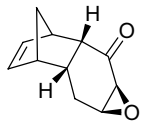
Freshly distilled cyclopentadiene (bp 42 °C; 15.3 mL, 0.185 mol) was added dropwise in 25 min to a solution of *p*-benzoquinone (20 g, 0.185 mol) in MeOH (100 mL) at 0-5 °C. The mixture was stirred for 2.5 h at room temperature. The brown liquid was diluted with MeOH to 460 mL and $CeCl_3.7H_2O$ (138 g, 0.370 mol) was added to the solution. The reaction mixture was cooled to 0-5 °C and $NaBH_4$ (14.0 g, 0.370 mol) was added slowly. The mixture was stirred for an additional 1 h at room temperature. The reaction was quenched with water, filtered extracted with EtOAc and dried (Na_2SO_4), and the solvent was evaporated. Recrystallization from acetone gave **2** (26 g, 80%) as pink crystals. Mp: 157 °C. IR υ_{max} film: (cm^{-1}) 3251, 2980, 2943, 2926, 1352, 1270, 1054. ^{1}H NMR (CD_3OD, 300 MHz, ppm): δ 5.76 (br s, 2H, H_4, and H_5), 5.25 (br s, 2H, H_9 and H_{10}), 4.39 (dd, J = 5.1, 12.6 Hz, 2H, H_3

and H_6), 3.00 (br s, 2H, H_1 and H_8), 2.80 (t, J = 3.3 Hz, 2H, H_2 and H_7), 1.29 (br s, 2H, H_{11}); in agreement with literature.[34]



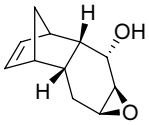
(±)-(1*R*,2*R*,7*R*,8*S*)-Tricyclo[6.2.1.0^{2,7}]undeca-4,9-dien-3-one (3)

To a solution of **2** (5.1 g, 29 mmol) and HCO_2NH_4 (2.7 g, 42 mmol) in degassed MeCN (280 mL), was added 1 mol% $PdCl_2$(dppf) (230 mg, 0.282 mmol). The solution was refluxed for 45-90 min. The reaction was diluted with Et_2O, washed with brine, and dried (Na_2SO_4). After evaporation of the solvent the crude product was purified by flash chromatography (EtOAc/*n*-heptane, 1/5) to give **3** (3.5 g, 77%) as a yellow oil. The reaction conditions were optimized with automated parallel syntheses performed with the Anachem synthesis robot (Gilson SK233).[35] ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 6.66 (dt, J = 4.0, 10.3 Hz, 1H, CH), 6.12 (ddd, J = 2.9, 5.6, 15.8 Hz, 2H, CH), 5.86 (dt, J = 10.4, 2.4 Hz, 1H, CH), 3.38 (br s, 1H, CH), 3.03 (br s, 1H, CH), 2.91 (dd, J = 10.1, 3.9 Hz, 1H, CH), 2.76 (dt, J = 10.1, 3.5 Hz, 1H, CH), 2.60 (dddd, J = 20.6, 10.2, 3.9, 2.4 Hz, 1H, CH), 2.02 (ddd, J = 20.5, 6.0, 3.4 Hz, 1H, CH), 1.42 (dt, J = 1.8, 8.4 Hz, 1H, CH), 1.34 (d, J = 8.4 Hz, 1H, CH); in agreement with literature.[36]



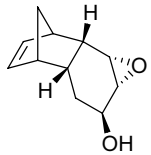
(±)-(1*R*,2*R*,4*S*,5*S*,7*R*,8*S*)- 4,5-Epoxytricyclo[6.2.1.0^{2,7}]undec-9-en-3-one (4)

35% H_2O_2 (8.6 mL) and 0.2 M aqueous NaOH (10.6 mL) were added to a solution of **3** (3.4 g, 21 mmol) in a mixture of CH_2Cl_2/MeOH (1:1, 200 mL) at room temperature. After the reaction had been stirred stirring for 30 min the reaction mixture was diluted with CH_2Cl_2 (300 mL). Washing with brine, drying (Na_2SO_4), gave the crude product which was purified by flash chromatography (EtOAc/*n*-heptane, 1/5), to obtain **4** (3.3 g, 89%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.22 (dd, J = 2.8, 5.5 Hz, 1H, H_{10}), 5.89 (dd, J = 5.6, 2.8 Hz, 1H, H_9), 3.42 (t, J = 4.1 Hz, 1H, H_4), 3.16 (dd, J = 1.0 4.5 Hz, 1H, H_2), 3.10 (br s, 1H, H_1), 2.90 (dd, J = 10.4, 3.4 Hz, 1H, H_5), 2.82-2.75 (m, 2H, H_{6exo}, and H_8), 2.38 (ddd, J = 14.7, 6.8, 3.6 Hz, 1H, H_7), 1.44 (d, J = 8.4 Hz, 1H, H_{11}), 1.33 (dd, J = 14.7, 10.8 Hz, 1H, H_{6endo}), 1.25 (d, J = 8.4 Hz, 1H, H_{11}). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 209.6, 138.5, 134.1, 57.6, 54.5, 50.6, 47.6, 45.5, 42.9, 36.3, 27.4; in agreement with literature.[36]



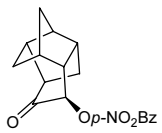
(±)-(1*R*,2*R*,3*S*,4*R*,5*S*,7*R*,8*S*)-4,5-Epoxytricyclo[6.2.1.0^{2,7}]undec-9-en-3-ol (5)

To a cold solution -78 °C of **4** (3.21 g, 18.2 mmol) in MeOH (46 mL) was added $NaBH_4$ (689 mg, 18.2 mmol) and $CeCl_3 \cdot 7H_2O$ (6.77 g, 18.2 mmol). The reaction was stirred until conversion was completed. The mixture was quenched with ammonium chloride, extracted with Et_2O, washed with brine and dried (Na_2SO_4). The crude mixture was purified by means of flash chromatography (EtOAc/*n*-heptane, 1/5 and 1% Et_3N) to yield (2.59 g, 80%) of compound **5** as colorless oil. R_f 0.4 (EtOAc/*n*-heptane, 1/3). IR υ_{max} film: (cm^{-1}) 3442, 2958, 1439, 1338, 1255, 816, 729. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.40 (dd, J = 5.7, 3.1 Hz, 1H, H_{10}), 6.06 (dd, J = 5.7, 3.1 Hz, 1H, H_9), 4.46 (m, 1H, H_3), 3.12 (m, 2H, H_4, and H_5), 2.93 (br s, 1H, H_1), 2.77 (br s, 1H, H_8), 2.46-2.38 (m, 1H, H_7), 2.30-2.24 (m, 2H, H_2, and H_{6exo}), 1.45 (dt, J = 8.1, 1.9 Hz, 1H, H_{11}), 1.34 (dd, J = 14.4, 11.7 Hz, 2H, H_{6endo}, and H_{11}), 1.11 (br d, 1H, OH). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 137.8, 134.0, 68.9, 51.8, 50.9, 50.7, 46.1, 45.7, 40.8, 36.1, 26.9. HRMS (CI) *m/z* calcd for $C_{11}H_{15}O_2$ $(M+H)^+$: 179.1072, found: 179.1068.



(±)-(1*R*,2*R*,3*S*,4*R*,5*S*,7*R*,8*S*)-5,6-Epoxytricyclo[6.2.1.0^{2,7}]undec-9-en-4-ol (6)

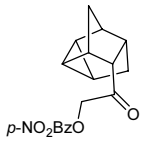
To a solution of **5** (20 mg, 0.11 mmol) in Et_2O (1.5 mL) was added NaH (2.7 mg, 0.11 mmol) at room temperature. The reaction was followed with TLC (EtOAc/*n*-heptane, 2/3). After stirring for 3 days water was added and the product was extracted with Et_2O, dried ($MgSO_4$). Analysis with TLC and NMR showed 100% conversion to **6**. R_f 0.30 (EtOAc/*n*-heptane, 1/3). IR υ_{max} film: cm^{-1} 3406, 2960, 1437, 1342, 1049, 735. ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): δ 6.23 (dd, J = 5.7, 2.9 Hz, 1H, H_{10}), 6.06 (dd, J = 5.7, 3.2 Hz, 1H, H_9), 4.23 (m, 1H, H_5), 3.25 (m, 1H, H_3), 3.00 (m, 2H, H_1 and H_4), 2.70 (br s, 1H, H_8), 2.47 (dt, J = 9.8, 2.6 Hz, 1H, H_2), 2.35-2.19 (m, 1H, H_7), 1.72-1.61 (m, 2H, H_{6exo} and OH), 1.42 (m, 1H, H_{11}), 1.29-1.21 (m, 2H, H_{11} and H_{6endo}). HRMS (CI) *m/z* calcd for $C_{11}H_{15}O_2$ $(M+H)^+$: 179.1072, found: 179.1071.



(±)-(1*S*,2*R*,4*R*,6*R*,7*R*,8*S*,9*R*)-10-Oxotetracyclo[5.3.1.0^{2,6}.0^{4,8}]undec-9-yl 4-nitrobenzoate (9)

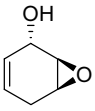
IR υ_{max} film: (cm^{-1}) 2959, 1724, 1527, 1269, 718. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.29 (d, J = 8.9 Hz, 2H, arom), 8.22 (d, J = 8.9 Hz, 2H, arom), 7.23 (s, 1H, CH), 5.61 (d, J = 6.0

Hz, 1H, CHOH), 3.00 (t, *J* = 6.0 Hz, 1H, CH), 2.79-2.51 (m, 3H, CH), 2.41 (s, 1H, CH), 1.85 (d, *J* = 13.0 Hz, 1H, CH_2), 1.71-1.62 (m, 3H, CH_2), 1.52-1.41 (m, 1H, CH_2), 1.37 (d, *J* = 10.2 Hz, 1H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 164.1, 131.1, 123.7, 74.5, 53.0, 48.2, 42.8, 38.8, 37.0, 36.9, 34.5. MS (CI) *m/z*: 328 $(M+H)^+$. Crystal structure has been deposited at the Cambridge Crystallographic Data Centre, CCDC 226045.



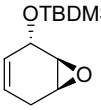
(±)-(1*R*,2*R*,3*R*,4*S*,6*R*,7*R*,8*R*)-2-Oxo-2-{tetracyclo[4.3.0.0^{2,4}.0^{3,8}]non-7-yl}ethyl 4-nitrobenzoate (12)

IR υ_{max} film: (cm^{-1}) 2961, 1720, 1526, 1346, 1275, 1100, 717. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.31-8.27 (m, 4H, arom), 5.50 (q, *J* = 6.0 Hz, 2H, CH_2), 2.74 (s, 1H, CH), 2.62 (s, 1H, CH), 2.61 (s, 1H, CH), 2.42 (d, *J* = 8.4 Hz, 1H, CH), 2.07 (d, *J* = 8.8 Hz, 1H, CH_2), 2.00-1.98 (m, 1H, CH), 1.88 (s, 2H, CH_2), 1.70-1.60 (m, 2H, CH_2, CH), 1.40-31 (m, 1H, CH). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 131.2, 123.7, 69.2, 55.0, 50.0, 46.1, 37.8, 33.1, 31.1, 30.2, 22.9. MS (CI) *m/z:* 328 $(M+H)^+$. Crystal structure has been deposited at the Cambridge Crystallographic Data Centre, CCDC 226044.



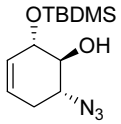
(±)-(1*S*,5*S*,6*R*)-5,6-Epoxycyclohex-2-en-1-ol (13)

The thermolysis oven was preheated to 600 °C. A solution of **5** (430, 2.41 mmol) in Et_2O was brought into the sublimation flask, and Et_2O was evaporated. The vacuum gauge was carefully opened until vacuum reached 0.04 mbar, after which the collecting cooler was charged with CO_2/acetone (-78 °C). The sublimation oven was heated to 80 °C at this stage. The reaction was finished when no starting material remained in the sublimation flask. The crude mixture was purified by flash chromatography (Et_2O/*n*-pentane, 1/2). Compound **13** (226 mg, 84%) was obtained as a colorless liquid. R_f 0.3 (EtOAc/*n*-heptane, 2/1). IR υ_{max} film: cm^{-1} 3390, 1419, 1011, 929, 986, 798, 710. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.7-5.66 (m, 1H, CH), 5.6-5.57 (m, 1H, CH), 4.48 (br s, 1H, CH), 3.31 (br s, 1H, CH), 3.25 (br s, 1H, CH), 2.63-2.48 (m, 2H, CH), 1.84 (br s, 1H, OH). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 124.8, 124.7, 63.0, 53.06, 50.2, 25.1.



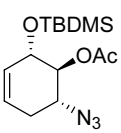
(±)-(3*S*,4*S*,5*S*)-3-[(*tert*-Butyldimethylsilyl)oxy]-4,5-epoxycyclohex-1-ene (14)

To a solution of **13** (655 mg, 5.84 mmol) in CH_2Cl_2 (30 mL) was added DIPEA (1.43 mL, 8.17 mmol), TBDMSCl (1.06 g, 7.59 mmol), and DMAP (71.0 mg, 0.584 mmol) at 0 °C. The solution was stirred 5 h at room temperature. Water was added, and the product was extracted with CH_2Cl_2, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc/*n*-heptane, 1/10), to yield **14** (1.12 g, 85%) as a colorless oil. R_f 0.6 (EtOAc/*n*-heptane, 1/3). IR υ_{max} film: (cm^{-1}) 2952, 2927, 2856, 1254, 1068, 837. ^1H NMR ($CDCl_3$, 400 MHz, ppm): 5.58-5.49 (m, 2H, 2CH), 4.49 (br s, 1H, CH), 3.30 (br s, 1H, CH), 3.15 (br s, 1H, CH), 2.54 (br s, 2H, CH_2), 0.92 (s, 9H, *t*-Bu), 0.14 (s, 3H, Me), 0.12 (s, 3H, Me). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 125.1, 123.2, 63.7, 54.1, 50.7, 25.9, 24.9, 18.3, -4.5, -4.6. HRMS (EI) *m/z* calcd for $C_{12}H_{22}O_2Si$ $(M)^+$: 226.1389, found: 226.1381. $C_{11}H_{19}O_2Si$ (M-Me): 211.1154, found: 211.1152.



(±)-(1*S*,2*S*,6*R*)-6-Azido-2-[(*tert*-butyldimethylsilyl)oxy]cyclohex-3-en-1-ol (15)

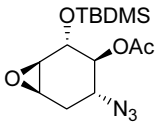
To a solution of **14** (3.05 g, 13.5 mmol) in MeOH (50 mL) was added NaN_3 (1.75 g, 26.9 mmol) and NH_4Cl (1.29 g, 24.1 mmol). The reaction was stirred under reflux for 40 h. MeOH was evaporated, CH_2Cl_2 was added, the solution was washed with brine, dried (Na_2SO_4). The solvent was evaporated and the crude product was purified by flash chromatography (EtOAc/*n*-heptane, 1/10), to obtain **15** as a colorless oil (4.2 g, 85%). IR υ_{max} film: (cm^{-1}) 2109, 1253, 1088, 837, 779. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.59-5.55 (m, 1H, CH), 5.50 (d, *J* = 10.1 Hz, 1H, CH), 4.22-4.20 (m, 1H, CH), 3.60-3.57 (m, 2H, 2CH), 2.50-2.44 (m, 1H, CH), 2.46 (s, 1H, OH, disappears with a drop of D_2O), 2.14-2.07 (m, 1H, CH), 0.91 (s, 9H, *t*-Bu), 0.13 (s, 3H, MeSi), 0.12 (s, 3H, MeSi). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 130.0, 124.1, 76.9, 73.9, 60.7, 31.0, 25.8, 18.1, -4.5. MS(CI): 270 (M+H). HRMS (EI) *m/z* calcd for $C_{12}H_{23}O_2SiN_3$ $(M)^+$: 269.1559, found: 269.1551.



(±)-(1*S*,2*S*,6*R*)-6-azido-2-[(tert-butyldimethylsilyl)oxy]-cyclohex-3-ene-5-olacetate (16)

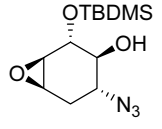
To a solution of **15** (63 mg, 0.234 mmol) in CH_2Cl_2 (2 mL), were added Ac_2O (44 µl, 0.468 mmol), Et_3N (65 µl, 0.468 mmol), and DMAP (cat.) at room temperature. The reaction was

stirred for 1 h. Brine was added and the product was extracted with CH_2Cl_2, and dried with Na_2SO_4. After evaporation of the solvent, the crude product was purified by flash chromatography (EtOAc/*n*-heptane, 1/10), to obtain **16** (56 mg, 77%). IR υ_{max} film: (cm^{-1}) 2929, 2102, 1754, 1253, 1222, 940, 837. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.64-5.59 (m, 1H, H-$_5$), 5.52 (d, *J* = 10.2 Hz, 1H, H-4), 5.06 (dd, *J* =10.9, 7.8 Hz, 1H, H-2), 4.34 (ddd, *J* = 7.7, 3.6, 1.8 Hz, 1H, H-3), 3.62 (td, *J* = 10.7, 5.9 Hz, 1H, H-1), 2.50 (dt, *J* = 5.4, 17.6 Hz, 1H, H-6a), 2.25-2.13 (m, 1H, H-6b), 2.15 (s, 3H, Ac), 0.87 (s, 9H, *t*-Bu), 0.09 (s, 3H, Me), 0.06 (s, 3H, Me). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 170.4, 130.6, 124.5, 71.7, 59.5, 31.3, 26.0, 21.5, 18.3, -4.2, -4.4. HRMS (CI) m/z calcd for $C_{14}H_{26}O_3SiN_3$ $(M+H)^+$: 312.1743, found: 312.1754.



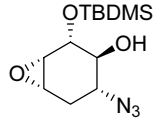
(±)-*(1R,2S,3S,4R,5R)*-1-azido-3-[(tert-butyldimethylsilyl)oxy]-4-epoxy-cyclohexane-2-ylacetate (17)

To a solution of **16** (37 mg, 0.12 mmol), and chlorocyclohexanone (0.12 mmol, 16 mg) in MeCN and water (3:2, 3 mL) was added in small portion during 1 hour oxone (0.12 mmol, 73 mg) and $NaHCO_3$ (0.36 mmol, 30 mg). The solution was stirred at room temperature for another hour and quenched with water, extracted twice with CH_2Cl_2, dried with Na_2SO_4, concentrated, and purified by flash chromatography to yield compound **17** as a colorless oil (27 mg, 71%). The ratio between *cis* and *trans*-epoxide is 1:4. IR υ_{max} film: (cm^{-1}) 2929, 2104, 1759, 1369, 1221, 1107, 841, 779. R_f 0.25 (EtOAc/*n*-heptane, 1/10). ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.84 (dd, *J* = 11.0, 8.1 Hz, 1H, H_2), 3.96 (d, *J* = 8.1 Hz, 1H, H-3), 3.41 (td, *J* = 11.1, 5.1 Hz, 1H, H-1), 3.30 (br s, 1H, H-5), 3.04 (d, *J* = 3.5 Hz, 1H, H-4), 2.56 (ddd, *J* = 14.8, 5.1, 2.0 Hz, 1H, H-6a), 2.13 (s, 3H, Ac), 1.90 (ddd, *J* = 14.8, 11.3, 1.6 Hz, 1H, H-6b), 0.89 (s, 9H, *t*-Bu), 0.14 (s, 3H, Me), 0.08 (s, 3H, Me). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 170.5, 71.5, 57.1, 56.0, 53.6, 30.5, 26.1, 21.6, 18.5, -4.1, -4.4. HRMS (CI) *m/z* calcd for $C_{14}H_{25}O_4SiN_3$ $(M+H)^+$: 328.1692, found: 328.1684.



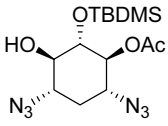
(±)-(1*S*,2*R*,3*R*,4*R*,6*R*)-6-Azido-2-[(*tert*-butyldimethylsilyl)oxy]-3,4-epoxycyclohexan-1-ol (19)

To a solution of **15** (3.61 g, 13.4 mmol) in CH_2Cl_2 (180 mL) at room temperature, was added *m*-CPBA (5.76 g, 33.4 mmol). After stirring overnight the suspension was diluted with CH_2Cl_2, filtered, washed with water, and twice with a phosphate buffer (pH 7.5) to get rid of the excess benzoic acid. The crude product was dried (Na_2SO_4), concentrated under reduced pressure, and purified by flash chromatography (EtOAc/*n*-heptane, 1/5) to give (2.74 g, 72%) of compound **19** as a colorless crystalline solid and (0.684 g, 18%) of compound **20**. Spectral data **19**: R_f 0.4 (EtOAc/*n*-heptane, 1/5). IR υ_{max} film: (cm^{-1}) 2956, 2927, 2860, 2110, 1709, 841. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.82 (d, 1H, 3, *J* = 7.2 Hz, CH), 3.42-3.32 (m, 3H, CH), 3.01 (d, 1H, *J* = 3.7 Hz, CH), 2.53 (ddd, *J* = 1.9, 3.1, 6.9 Hz, 1H, CH_2), 2.40 (d, *J* = 2.6 Hz, 1H, OH), 1.79 (ddd, *J* = 1.3, 10.4, 11.9 Hz, 1H, CH_2) 0.93 (s, 9H, *t*-Bu), 0.18 (s, 3H, MeSi), 0.16 (s, 1H, MeSi). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 73.2, 57.4, 56.4, 53.1, 28.9, 25.9, 18.2, -4.5, -4.6. HRMS (CI) *m/z* calcd for $C_{12}H_{24}O_3SiN_3$ $(M+H)^+$: 286.1587, found: 286.1577.



(±)-(1*S*,2*R*,3*S*,4*S*,6*R*)-6-Azido-2-[(*tert*-butyldimethylsilyl)oxy]-3,4-epoxycyclohexan-1-ol (20)

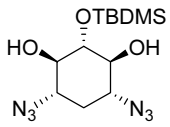
R_f 0.3 (EtOAc/*n*-heptane, 1/5). IR υ_{max} film: (cm^{-1}) 2954, 2105, 1769, 1374, 1247, 837, 780, 608. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.76 (d, *J* = 8.1 Hz, 1H), 3.44 (t, *J* = 9.8 Hz, 1H), 3.20 (q, *J* = 10.6, 18.2 Hz, 1H), 3.08-2.98 (m, 2H), 2.24-2.14 (m, 1H), 1.75-1.65 (m, 1H), 0.78 (s, 9H), 0.01 (s, 6H). ^{13}C NMR (CD_3OD, 100 MHz, ppm): δ 75.2, 62.4, 58.8, 52.4, 30.7, 26.5, 19.2, -3.9, -4.4. HRMS (CI) *m/z* calcd for $C_{12}H_{24}O_3SiN_3$ $(M+H)^+$: 286.1587, found: 286.1577.



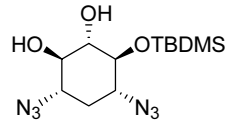
(±)-(1*R*,2*S*,3*S*,4*S*,6*R*)-4,6-Diazido-2-[(*tert*-butyldimethylsilyl) oxy]cyclohexane-3-acetyl-1,3-diol (22)

To a solution of **17** (40 mg, 0.14 mmol) in MeOH (2 mL) was added NaN_3 (26 mg, 0.56 mmol) and NH_4Cl (18 mg, 0.50 mmol). The reaction mixture was refluxed for 48 h. and the solvents were evaporated. The crude was dissolved in EtOAc and washed with H_2O several times. The crude product was dried (Na_2SO_4), concentrated under reduced pressure, and purified by flash chromatography (EtOAc/*n*-heptane, 1/10) to give (9 mg, 21%) of compound **19** as a colorless oil. The wrong regio isomer was also isolated as a colorless oil (32 mg, 74%). Spectral data **19***: R_f* 0.06 (EtOAc/*n*-heptane, 1/10). IR υ_{max}

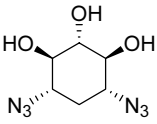
film: (cm^{-1}) 2932, 2357, 2103, 1748, 1376, 1255, 841. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.88 (t, 1H, J = 9.2 Hz), 3.66-3.31 (m, 4H), 2.41 (s, 1H), 2.31 (m, 1H), 2.11 (s, 3H), 1.45 (dt, 1H), 0.88 (s, 9H), 0.16 (s, 3H), 0.11 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 168.8, 74.7, 74.0, 59.3, 58.3, 31.4, 25.3, 20.8, 17.7, -4.3, -4.9. HRMS (CI) *m/z* calcd MS $C_{14}H_{26}O_4N_6Si$ $(M+H)^+$: 371.1864, found: 371.1863.



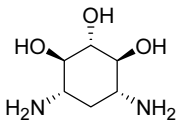
(1*R*,2*r*,3*S*,4*R*,6*S*)-4,6-Diazido-2-[(*tert*-butyldimethylsilyl) oxy]cyclohexane-1,3-diol (24)
A solution of the epoxide (**19**) (448 mg, 1.66 mmol) in 22 mL toluene is added dropwise under argon over $Yb(OTf)_3$ (515 mg, 0.83 mmol) and MS 4Å (500 mg) at room temperature. NaN_3 (1.08 g, 16.6 mmol) and Et_3N (3.47 ml, 24.9 mmol) are added and the reaction is stirred at 80 °C for 4 days. The reaction mixture is cooled, filtered and evaporated. The crude product was purified by flash chromatography (EtOAc/*n*-heptane, 1/10), to give compound **24** as white crystals (321 mg, 82% based on recovered starting material 114 mg). R_f 0.2 (EtOAc/*n*-heptane, 1/10). Mp: 104 °C. IR υ_{max} film: (cm^{-1}) 2932, 2098, 1247, 1130, 841. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.35 (s, 5H), 2.41 (s, 2H, OH), 2.18 (m, 1H), 1.38 (m 1H), 0.92 (s, 9H), 0.16 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 76.2, 59.7, 31.7, 25.7, 18.1, -4.5.HRMS (CI) *m/z* calcd for $C_{12}H_{25}O_3SiN_6$ $(M+H)^+$: 329.1758, found: 329.1754. Anal. calcd for $C_{12}H_{24}O_3N_6Si$: C 43.88, H 7.37, N 25.59; found: C 43.84, H 7.04, N 25.11. Crystal structure have been deposited at the Cambridge Crystallographic Data Centre, CCDC 226046.



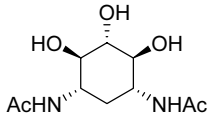
(±)-(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-Diazido-1-[(*tert*-butyldimethylsilyl)oxy]cyclohexane-2,3-diol (26)
Compound **24** (30 mg, 0.0 93 mmol) was dissolved in THF (1mL). The mixture was cooled to 0 °C and TBAF (1 M solution, 100 µL) was added. After stirring for 30 minutes the reaction mixture was diluted with Et_2O, filtered, washed with water, and brine. The crude product was dried (Na_2SO_4), concentrated under reduced pressure, and purified by flash chromatography (EtOAc/*n*-heptane, 1/10) to give (30 mg, quant.) of compound **26** as a colorless oil. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.45-3.25 (m, 5H), 2.86 (br s, 1H, OH), 2.51 (br s, 1H, OH), 2.43-2.12 (m, 1H, CH_2), 1.45-1.12 (m, 1H, CH_2), 0.93 (s, 9H, *t*-Bu), 0.19 (s, 3H, Me), 0.15 (s, 3H, Me).



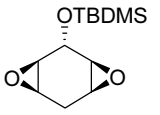
(1*R*,2*r*,3*S*,4*R*,6*S*)-4,6-Diazidocyclohexanetriol (27)
Compound **24** (30 mg, 0.093 mmol) was dissolved in a 1N HCl solution in MeOH (1 mL). The reaction mixture was stirred at room temperature overnight. EtOAc was added and the reaction mixture was washed with $NaHCO_3$ and dried (Na_2SO_4), to give after flash chromatography (EtOAc) the 4,6-diazidocyclohexanetriol (quant.). R_f 0.4 (EtOAc). IR υ_{max} film: (cm^{-1}) 3369, 2923, 2100, 1359, 1260, 1113, 1080, 1023, 668, 616, 556. ^1H NMR (CD_3OD, 400 MHz, ppm): δ 3.38 (m, 2H, CH), 3.18-3.27 (m, 3H, CH), 2.09 (dt, J = 4.4 Hz, 1H, CH_2), 1.25 (q, J = 12.6 Hz, 1H, CH_2); in agreement with literature.[37]



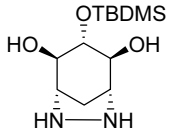
2-Deoxystreptamine
To a solution of 4,6-diazidocyclohexanetriol **27** (20 mg, 0.093 mmol) in MeOH was added Pd/C (spatula). After the mixture had been stirred for 14 h under 3 bar of H_2, Pd/C was filtered off and the filtrate was concentrated to yield 2-deoxystreptamine (14 mg, 95%). IR υ_{max} film: (cm^{-1}) 3345, 2917, 2362, 2094, 1559, 1541, 1095, 988. ^1H NMR (CD_3OD, 400 MHz, ppm): δ 3.13 (m, 1H, CH), 3.02 (t, J = 9.5 Hz, 2H, CH), 2.68-2.54 (m, 2H, 2CH), 1.98 (dt, J = 4.3, 4.1 Hz, 1H, CH_2) 1.16 (q, J = 12.1 Hz, 1H, CH_2).[38]



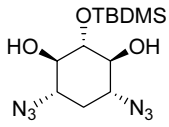
***N*,*N'*-diacetyl-2-deoxystreptamine (28)**
2-Deoxystreptamine (0.086 mmol, 14 mg) was dissolved in MeOH/H_2O (1/1) and Ac_2O (0.172 mmol, 16 µl) and Et_3N (0.172 mmol, 24 µl) were added. The reaction mixture as stirred for 3 h and then concentrated in vacuo and co-evaporated a few times with D_2O, to yield the diacetyl compound (20 mg, 96%). ^1H NMR (D_2O, 400 MHz, ppm): 3.82-3.71 (m, 2H, CH), 3.49-3.38 (m, 3H, 3CH), 2.03 (dt, J = 4.3 Hz, 1H, CH_2), 2.01 (s, 6H, CH_3), 1.42 (q, J = 12.5 Hz, 1H, CH_2). ^{13}C NMR (D_2O, 75 MHz, ppm): δ 174.8, 77.0, 75.5, 50.9, 33.8, 23.4; in agreement with literature.[39]



(1*R*,2*R*,3*r*,4*S*,5*S*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1,2-4,5-disepoxycyclohexane (29)
Compound **14** (50 mg, 0.22 mmol) was dissolved in CH_2Cl_2 (2 mL) *m*-CPBA (56 mg) and $NaHCO_3$ (18 mg) were added to the reaction mixture. The reaction was stirred at room temperature for 48 hours. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was washed twice with a phosphate buffer solution, dried with Na_2SO_4 and the solvents were evaporated. Two diastereomers xa (*cis*) and xb (*trans*) were formed in a ratio of 9:1. Both products were separated by column chromatography (EtOAc/*n*-heptane 1/10), to yield compound **29** (37 mg, 70%) as a white powder: R_f 0.16 (EtOAc/*n*-heptane, 1/5), Mp 85.2 °C. IR υ_{max} film: (cm^{-1}) 2956, 2929, 2856, 2358, 1739, 1438, 1257, 1095, 1012, 802. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.53 (s, 1H, CH), 3.12 (brs, 2H, epoxide), 3.01 (br s, 2H, epoxide), 2.67 (dd, *J* = 0.9, 16.4 Hz, 1H), 2.32 (dd, *J* = 3.1, 6.0 Hz, 1H), 0.94 (s, 9H, *t*Bu), 0.17 (s, 6H, Me). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 67.4, 57.5, 54.6, 30.6, 23.1, 23.0, 0.01. CI/MS *m/z* $(M+C_2H_5)^+$ 271. Anal. calcd for $C_{12}H_{22}O_3Si$: C 59.46, H 9.15; found: C 59.30, H 9.11.



(1*R*,2*S*,3*r*,4*R*,5*S*)-3-[(*tert*-Butyldimethylsilyl)oxy]-6,7-diazo-dicyclo[3,2,1]octane (30)
Compound **29** (50 mg, 0.21 mmol) was dissolved in EtOH (5 mL) and hydrazine (50 µL, 1.6 mmol) was added. The reaction was refluxed for 4 hours and the solvent was removed under reduced pressure to obtain **30**. R_f 0.0 (EtOAc/*n*-heptane, 1/3). ^{1}H NMR (CD_3 OD, 400 MHz, ppm): δ 3.60 (s, 1H, CH), 3.59 (s, 2H, CH), 3.42 (br s, 2H, CH), 2.70 (d, 1H, CH_2), 1.47 (dt, *J* = 6.0 Hz, 1H, CH_2), 0.94 (s, 9H, *t*Bu), 0.15 (s, 6H, Me). HRMS (EI) *m/z* calcd MS $C_{12}H_{26}O_3N_2Si$ $(M)^+$: 274.1713, found: 274.1710.

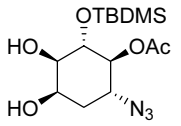


(1*R*,2*r*,3*S*,4*R*,6*S*)-4,6-Diazido-2-[(*tert*-butyldimethylsilyl) oxy]cyclohexane-1,3-diol (24)
Dissolve **30** (54 mg, 0.20 mmol) in MeOH/H_2O, 1/1 (30 mL). Amberlite IRA 400 (OH^-) (~20 mg) and Raney Nickel 2800 catalyst (~1.5 mL, slurry) are added. This is placed under an atmosphere of 3 bar H_2 for 16 hours. Filtrate the suspension over Celite and remove the solvent under reduced pressure. The crude compound (55 mg, 0.20 mmol) and $ZnCl_2$ (cat) were dissolved in H_2O (1.5 mL). Et_3N (170 µL, 0.60 mmol) and carefully MeOH (5.0 mL) were added. Followed by addition of a 0.6 M TfN_3 in CH_2Cl_2 (1.5 mL). The reaction mixture was stirred for three hours after which the solvents carefully evaporated. Purify the product by flash column chromatography (EtOAc/*n*-heptane, 1/10) to obtain **1** (6 mg, 10% in three steps). R_f 0.6 (EtOAc/*n*-heptane, 1/3). Spectral data in agreement with pervious.

Dihydroxylation with OsO_4
Compound (**16**) was dissolved in a mixture of H_2O and acetone (2/1, 0.1 M solution). To this mixture was added NMO (1equiv) and OsO_4 (1 mol %- 100 mol %). The mixture was stirred at room temperature.

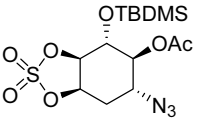
Dihydroxylation with OsO_4 and methanesulfonamide
OsO_4 (0.2 mL mg, 0.03 mmol) was dissolved in a mixture of H_2O and *t*-BuOH (1/1, 2 mL solution) and methanesulfonamide (3 mg, 0.03 mmol). To this mixture was added compound (**16**) (10 mg, 0.032 mmol). The mixture was stirred at room temperature for 5h, solid sodium sulfite was added and the mixture was stirred for 30 min. EtOAc was added to the reaction mixture, and after separation of the layers, the aqueous phase was further extracted with the EtOAc (3 X 5 mL). The combined organic extracts were dried with Na_2SO_4 and concentrated to give the diol and the ligand. This crude product was purified by flash chromatography (EtOAc/hexanes; 1/5) to afford the 1,2-diol **31** (12 mg, quant.).

Dihydroxylation with the $NaIO_4$
To a cold 0 °C solution of EtOAc and MeCN (1/1, 1 mL) was added compound **16** (60 mg, 0.19 mmol) and $RuCl_3.H_2O$ (2.7 mg, 0.013 mmol) and $NaIO_4$ (56 mg, 0.28 mmol) in H_2O (1 mL). The reaction was stirred for 3 min and quenched with saturated $Na_2S_2O_3$, extracted twice with EtOAc, dried with Na_2SO_4, evaporate the solvents, and purify by flash chromatography (EtOAc/*n*-heptane, 1/3) to yield compound **31** as a colorless oil (59 mg, 88%).



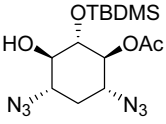
(±)-(1*R*,2R,3*S*,4*S*,5*R*)-5-azido-3-[(*tert*-butyldimethylsilyl)oxy]cyclohexane-4-acetyloxy-1,2-diol (31)

R_f 0.36 (EtOAc/*n*-heptane, 2/3). IR υ_{max} film: (cm^{-1}) 2928, 2111, 1722, 1238, 836. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.87 (t, *J* = 9.8 Hz, 1H, CH), 4.13 (br s, 1H, CH) 3.86 (t, *J* = 9.2 Hz, 1H, CH), 3.52 (dq, *J* = 2.2, 5.6 Hz, 1H, CH), 3.49 (dt, *J* = 2.6, 3.1 Hz, 1H, CH) 2.48 (br s, 1H, OH), 2.31 (br s, 1H, OH), 2.29 (dt, *J* = 3.7 Hz, 1H, CH_2), 2.25 (s, 3H, Me), 1.61 (m, 1H, CH_2), 2.15 (s, 9H, *t*-Bu), 0.13 (s, 3H, Me), 0.09 (s, 3H, Me). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 169.9, 76.2, 75.3, 72.7, 67.9, 58.3, 32.8, 26.0, 21.6, 18.4, -3.8, -4.0. HRMS (CI) *m/z* calcd MS $C_{14}H_{27}O_5N_3Si$ $(M+H)^+$: 346.1803, found: 346.1798.



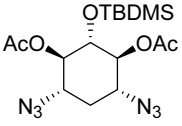
(±)-(1*R*,2S,3*R*,4*S*,5*R*)-5-azido-1,2-sufate-3-[(*tert*-butyldimethylsilyl)oxy]cyclohexane-4-acetyloxy-1,2-diol (32)

To a solution of the diol (50 mg, 0.14 mmol) and thionyl chloride (18 µl, 0.14 mmol) in dry EtOAc was added pyridine (25 µl, 0.29 mmol). The mixture was stirred and not allowed to rise above rt. When TLC analysis showed complete conversion the mixture was diluted with EtOAc and extracted with H_2O. The organic phase was dried with Na_2SO_4 and concentrated. The residue was dissolved in a mixture of CH_2Cl_2, MeCN and H_2O (2:2:3, 1 mL), $NaIO_4$ (61 mg, 0.28 mmol) and $RuCl_3 \times H_2O$ (catalytic) were added and the mixture was stirred for 1h at 20 °C. The mixture was filtered and the filtrate was diluted with CH_2Cl_2, the organic layer was extracted with water, dried ($MgSO_4$) and concentrated. Flash column chromatography (EtOAc/*n*-heptane, 1/5) yielded compound **32** as a white solid. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.24 (m, 1H), 4.91 (t, *J* = 9.7 Hz, 1H), 4.72 (dd, *J* = 4.8, 7.6 Hz, 1H), 4.24 (dd, *J* = 7.6, 9.5 Hz, 1H), 3.80 (m, 1H), 2.57-2.23 (m 1H), 2.17 (s, 3H) 1.89 (dt, *J* = 3.7 Hz, 1H), 0.86 (s, 9H), 0.16 (s, 3H), 0.10 (s, 3H). HRMS (CI) *m/z* calcd MS $C_{14}H_{25}O_7N_3SiS$ $(M+H)^+$: 408.1261, found: 408.1257.



(±)-(1*R*,2*S*,3*S*,4*R*,6*S*)-4,6-Diazido-2-[(*tert*-butyldimethylsilyl) oxy]cyclohexane-3-acetyloxy-1-ol (22)

Compound **32** (18 mg, 0.044 mmol) was dissolved in DMF (1 mL). The reaction mixture was heated top 80 °C and LiN_3 (18 mg, 0.11 mmol) was added to the reaction mixture. The reaction mixture was heated until TLC analysis showed conversion of the cyclic sulfate into baseline material. After stirring for 2 h, the reaction mixture was evaporated and dissolved in THF. A drop of water was added and H_2SO_4 (5 µL) was added to the reaction mixture. The reaction mixture was quenched after 30 min. with $NaHCO_3$ and extracted with EtOAc, dried with Na_2SO_4 and purified by flash column chromatography (EtOAc/*n*-heptane, 1/5) gave (23 mg, 43% for 2 steps) as an oil. Spectra in agreement with previous.



(1*R*,2*r*,3*S*,4*R*,6*S*)-4,6-Diazido-2-[(*tert*-butyldimethylsilyl) oxy]cyclohexane-1,3-acetyldiol (33)

Compound **22** (13 mg, 0.035 mmol) was dissolved in CH_2Cl_2 (0.8 mL) and add Et_3N (8 µL, 0.07 mmol) and Ac_2O (8 µL, 0.07 mmol) and DMAP (cat). Stir for 3 hours, dilute with CH_2Cl_2, wash with brine and dry with Na_2SO_4. Evaporation of the organic solvent and purification by flash column chromatography (EtOAc/*n*-heptane, 1/5) yields **33** as colorless oil (13 mg, 87%).

Compound **24** (100 mg, 0.30 mmol) was dissolved in CH_2Cl_2 (3 mL) and Et_3N (84 µL, 0.75 mmol), Ac_2O (72 µL, 0.75 mmol) and DMAP (cat) were added. The reaction mixture was stirred for 2 hours, diluted with CH_2Cl_2, washed with brine and dried with Na_2SO_4. Evaporation of the organic solvent and purification by flash column chromatography (EtOAc/*n*-heptane, 1/10 to 1/5) yields **33** as colorless oil (117 mg, 93%).

33: R_f 0.24 (EtOAc/*n*-heptane, 1/5). IR υ_{max} film: (cm^{-1}) 2951, 2930, 2857, 2098, 1746, 1372, 1250, 1215, 1144, 1096, 1067, 1032, 979, 896, 878, 836, 779. ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 4.97 (dd, *J* = 9.2, 10.2 Hz, 2H), 3.69 (t, *J* = 9.3 Hz, 1H), 3.40 (ddd, *J* =12.5, 10.2, 4.5 Hz, 2H), 2.30 (td, *J* = 4.5, 13.1 Hz, 1H), 2.17 (s, 6H), 1.62 (dd, *J* = 12.6, 25.8 Hz, 1H), 0.85 (s, 9H), 0.08 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 169.6, 77.4, 77.1, 76.8, 75.7, 72.2, 58.7, 31.8, 25.5, 22.8, 21.3, 17.8, -4.3. HRMS (EI) *m/z* calcd for $C_{16}H_{29}O_5SiN_6$ $(M+H)^+$: 413.1969, found: 413.19706.

Table 4. Crystallographic data and parameters for compounds **9**, **12**, **24** and **29**

Compound	**9**	**12**	**24**	**29**
Empirical formula	$C_{18}H_{17}NO_5$	$C_{18}H_{17}NO_5$	$C_{12}H_{24}N_6O_3Si$	$C_{12}H_{22}O_3Si$
Molecular mass	327.33	327.33	328.46	242.39
Temperature, K	208(2)	208(2)	208(2)	293(2)
Wavelength, Å	0.71073	0.71073	0.71073	0.71073
Crystal system	triclinic	monoclinic	monoclinic	monoclinic
Space group	P-1	C2/c	P 21/c	P 21/a
Unit cell dimension (Å, °)				
A	7.0838(4)	41.943(3)	11.7487(7)	12.565(4)
B	14.5357(7)	7.1885(6)	8.6317(7)	7.882(3)
C	16.0758(9)	10.2993(8)	17.4180(12)	14.321(6)
α	71.249(5)	90.000	90	90
β	78.019(5)	97.023(7)	92.494(6)	90.80(3)
γ	78.019(5)	90.000	90	90
Volume ($Å^3$)	1532.27(14)	3082.0(4)	1764.7(2)	1418.1(9)
Z	4	8	4	4
Density (calculated, mg/m^3)	1.419	1.411	1.236	1.135
μ, mm^{-1}	0.104	0.104	0.154	0.158
F(000)	688	1376	704	528
Crystal size, mm	0.19x0.15x0.03	0.26x0.26x0.03	0.26x0.14x0.06	0.29 x0.20x0.12
θ range (°)	3.53 - 27.50	3.19 - 27.50	3.70 - 27.50	5.01 - 27.54
Reflections collected	43104	41867	36090	20820
Independent reflections [R_{int}]	7002 [0.1121]	3541 [0.1077]	4034 [0.1059]	3248 [0.0597]
Refinement method		Full-matrix least-squares on F^2		
Data/restraint/parameters	7002 / 0 / 569	3541 / 0 / 278	4034 / 0 / 240	3248 / 0 / 233
Goodness-of-fit on F^2	1.025	1.029	1.081	1.032
R_1, ωR_2 indices	0.0608, 0.0967	0.0685, 0.1520	0.0830, 0.1740	0.0606, 0.1200
R_1, ωR_2 indices (all data)	0.1413, 0.1162	0.1378, 0.1799	0.1509, 0.2030	0.1094, 0.1378
Largest diff peak and hole, e. A^{-3}	0.200 and -0.254	0.532 and -0.244	0.987 and -0.271	0.241 and -0.180

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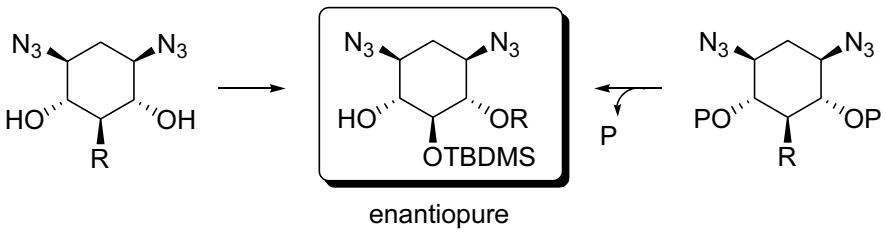
4 Desymmetrization of 1,3-diazidocyclohexitols

Abstract

Several methodologies are explored to obtain enantiopure and orthogonally protected 1,3-diazidocyclohexitols (1,3-DACHs), *via* resolution of a racemic intermediate of the synthetic route or *via* resolution of a *meso* 5-*O*-protected precursor, for example by asymmetric alkylation, asymmetric allylic alkylation or enzymatic resolution. A straightforward synthesis of 5-*O*-benzyl protected 1,3-diazido-4,5,6-cyclohexanetriol is developed, starting from commercially available kanamycin.



4.1 Introduction

The core structure of all clinically important aminoglycoside antibiotics is formed by an aminocyclitol termed streptamine. In particular, a monodeoxygenated version of streptamine, termed 2-deoxystreptamine or 2-DOS, features in natural or semi-synthetic aminoglycosides and as such appears indispensable for the development of any new aminoglycoside-type antibiotic or, more generally speaking, for RNA-specific high affinity ligands. Indeed, recent structural studies toward novel aminoglycoside entities with omission of 2-DOS led to low bacterial activity.[1] An important reason therefore to synthesize 2-DOS is that it may serve as a worthwhile scaffold in the development of new and innovative antibiotics.

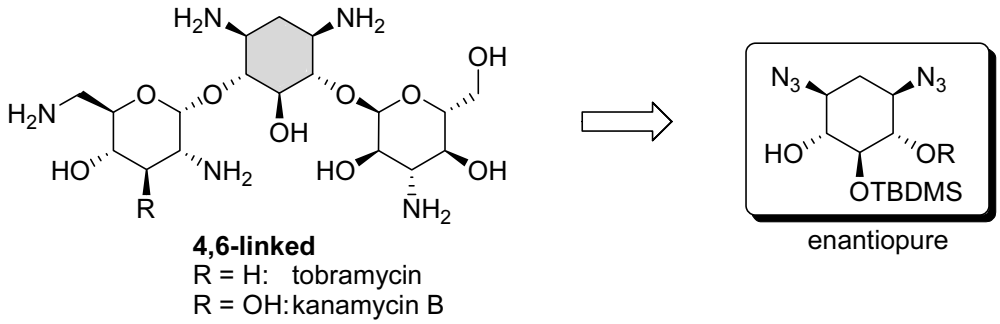


Figure 1. Asymmetrically substituted 2-deoxystreptamine in two common aminoglycosides.

In the preceding chapter we have synthesized two different 1,3-diazidocyclohexitol (1,3-DACH) precursors, **1** and **2**. Cyclic sulfate methodology yielded the racemic 2-DOS precursor **2**, whereas the other *meso* 2-DOS precursor **1** was obtained *via* a more straightforward strategy, by epoxide opening. This chapter describes the exploration of several methods to desymmetrize the 2-DOS precursors **1** and **2**, with particular attention on methodology to desymmetrize *meso* diol **1**, since in theory a 100% yield with 100% enantiomeric excess can be achieved starting from a *meso* compound.

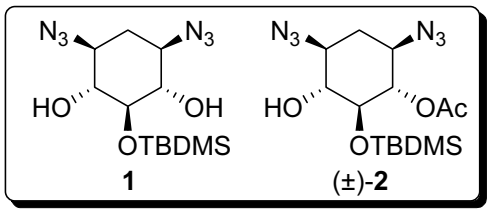
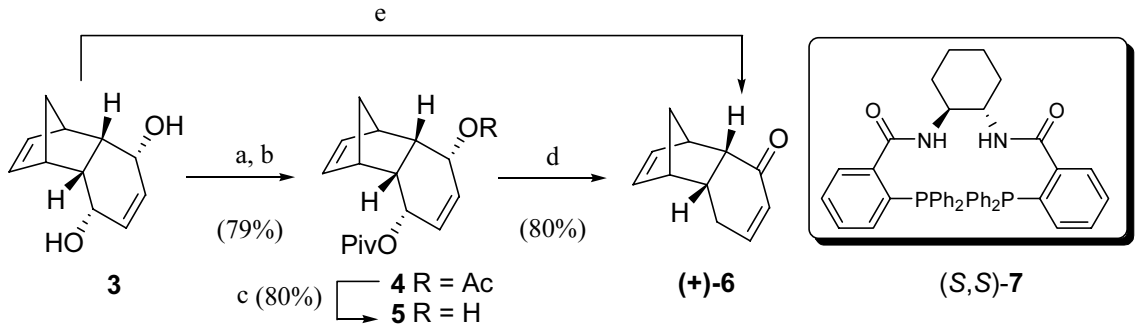


Figure 2. 1,3-DACHs **1** and **2** synthesized in chapter 3.

4.2 Asymmetric palladium-catalyzed 1,4-hydrogen migration

In the synthetic route toward 2-DOS described in chapter 3, the earliest occasion for desymmetrization of an intermediate lies in the resolution of ene-1,4-diol **3** (Scheme 1), prepared in two steps from 1,4-benzoquinone and cyclopentadiene. As described by Takano and co-workers, *meso* diol **3** can be converted into the enantiopure tricyclic enone **6** –the proper enantiomer for our purpose (*vide infra*)– in a few steps (Scheme 1):[2,3] first enantioselective acetylation with lipase PS, then protection of the remaining hydroxyl with a pivaloyl group (**3**→**4**), followed by selective deacetylation (**4**→**5**) and finally palladium-mediated hydrogen migration to yield enantiopure ketone **6** in an overall yield of 51% for the 4 steps.[4]

Scheme 1.



Reagents and conditions: (a) lipase PS, vinylacetate, MeCN, 30 °C; (b) PivCl, DMAP, Et_3N, CH_2Cl_2; (c) K_2CO_3, MeOH; (d) $Pd_2Cl_2(PPh_3)_2$, HCO_2NH_4, MeCN; (e) given Pd source, Trost ligand (*S,S*)-**7**, MeCN.

We reasoned that ketone **6** could possibly also be obtained in enantioselective fashion by a 1,4-hydrogen migration in the presence of a chiral ligand (Scheme 1),[5] particularly since catalysis of palladium-mediated reactions with enantiopure phosphine ligands is precedented. Thus, in an initial attempt compound **3** was subjected to the isomerization conditions ($Pd(dba)_2$, MeCN, 60 °C) explored in chapter 3, in the presence of Trost ligand (*S,S*)-**7**. In our first attempt, enantiopure ketone (+)-**6** was obtained in moderate yield (45%) but with excellent enantiomeric excess (97%).[6] However, besides (+)-**6** formation of another product was observed, in a ratio of 45:55 in favor of the side-product (Table 1, entry 1). Unfortunately, the side-product could not be isolated possibly due to instability. A few different reaction conditions were investigated but since none of the attempts led to an improval of product yield (entry 2-4), this methodology was abandoned.

Table 1. Enantioselective Pd-catalyzed 1,4-hydrogen migration

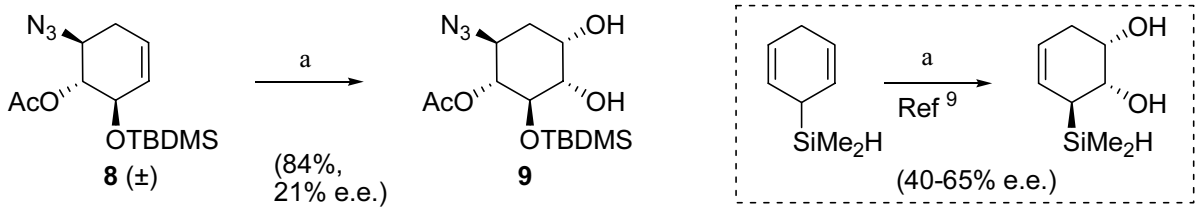
	hydrogen donor	catalyst	temperature	time	(+)-**6** yield (%)[a]	u.c.[b] yield (%)[a]
1	-	$Pd(dba)_2$	60 °C	3 h	45	55
2	-	$Pd(dba)_2$	Δ	40 min	45	55
3	HCO_2NH_4	$Pd(OAc)_2$	60 °C	3 h	23	77
4	-	$PdCl_2(C_3H_5)_2$	Δ	30 min	28	72

[a] GC yield [b] unidentified compound

4.3 Asymmetric dihydroxylation

In the early 90's Sharpless *et al.* developed a highly useful protocol for the asymmetric dihydroxylation (ADH) of a wide variety of olefines. The discovery of both the phthalazine class of ligands[7] and the acceleration of osmate ester hydrolysis of organic sulfonamides have led to one general procedure for diol formation from a wide range of alkenes with good to excellent enantiomeric excesses. Since our synthetic route toward 2-DOS involved the dihydroxylation of an alkene (*vide* chapter 3), asymmetric dihydroxylation was explored as a possible means to obtain an enantiopure 2-DOS precursor. Thus, the double bond of intermediate **8** was reacted under Sharpless asymmetric dihydroxylation conditions[8] with AD-mix β, to yield the dihydroxylated product **9** in a satisfactory yield of 84% (BORSM 96%), but with disappointing enantiomeric excess (21%) as determined by HPLC.[7] Because no proof of absolute stereochemistry could be obtained, the stereochemical constitution was assigned by analogy (Scheme 2).[9] The rather low e.e. of the ADH stimulated us to investigate whether double stereodifferentiation was playing a role and, consequently, if dihydroxylation under the action of AD-mix α would lead to a significantly different e.e. However, also upon reaction with AD-mix α product **9** was isolated in an enantiomeric excess of only 9% (yield 71%, BORSM 89%). Since these findings strongly suggest that the facial selectivity of the dihydroxylation is predominantly determined by the constitution of the starting material this route was further discarded.

Scheme 2.



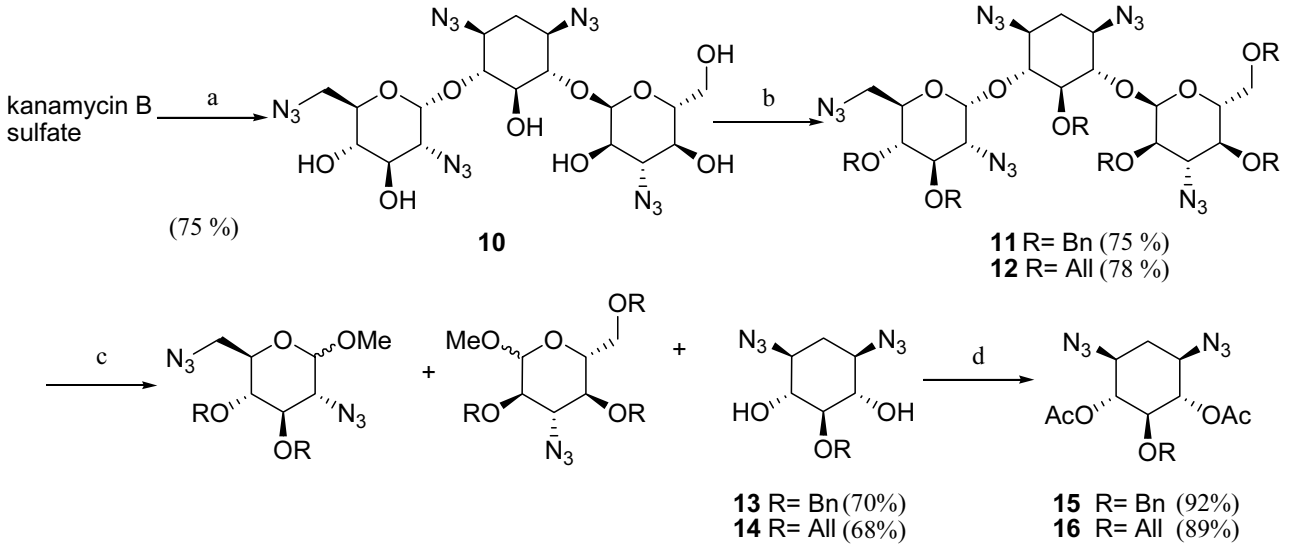
Reagents and conditions: AD-mix, $CH_3SO_2NH_2$, *t*-BuOH/H_2O (1/1), 0 °C, 24h, AD-mix β; 84% yield, 21% e.e., AD-mix α; 71% yield, 9% e.e.

4.4 1,3-DACHs from kanamycin

So far, attempts to obtain an enantioselective, protected 2-DOS precursor were based on the synthetic route described in the previous chapter. The latter route (§3.3), a 10-step sequence from cheap starting materials, was amenable to scale-up to afford several grams of 2-DOS in a few weeks time. Nevertheless, by far the simplest procedure to obtain 2-DOS remains the hydrolytic degradation of a natural aminoglycoside. Generally neomycin B trisulfate, since it is readily obtained by *Streptomyces* fermentation.[10] Thus, acidic degradation of neomycin yields 'naked' 2-DOS in an overall yield of 40% in two steps.[11] However, since neomycin is an aminoglycoside 4,5-linked at 2-DOS it is not well suitable for the preparation of 4,6-protected 2-DOS analogues. The synthesis described in this paragraph therefore starts from commercially available kanamycin B sulfate (5.20 €/mmol) which is more costly than neomycin,[12] but as will become clear below, can be converted into versatile scaffolds **13** or **14** in three straightforward steps.

The first step in our synthesis is a Zn-catalyzed diazotransfer onto kanamycin B, to give the corresponding azides, according to Wong and co-workers.[13] The free alcohols of the resulting tetraazide **10** were protected with an allyl or benzyl protective group, to give fully protected trisaccharide **11** or **12**, respectively. Subsequent acetal hydrolysis was performed with hydrochloric acid (Scheme 3) to afford the benzyl or allyl protected DACHs **13** and **14** in an overall yield of 39% and 40%, respectively. Other methods were also investigated *i.e.* hydrobromic acid, sulfuric acid and Amberlite IR-120 (H^+), but did not lead to satisfactory yields of the desired products.

Scheme 3.



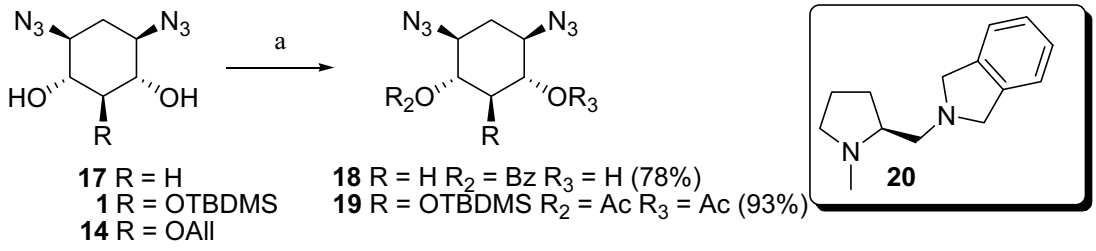
Reagents and conditions: (a) TfN_3, $ZnCl_2$, Et_3N, CH_2Cl_2/MeOH/H_2O, 3/10/3, rt, 3 h; (b) NaH, TBAI, AllBr or BnBr, DMF, 0 °C-rt, 16 h; (c) 1 N HCl/MeOH, Δ, 16 h; (d) Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt, 3 h.

Since both compounds **13** and **14** are *meso*, an important advantage of the route described here is that the obtained diazido entities can be directly subjected to enzymatic resolution. Moreover, the diazido derivatives described here display distinct advantages over carbamate-protected 2-DOS, as reflected in excellent solubility in organic solvents and straightforward spectral analysis.

4.5 Enantioselective acylation of 1,3-DACHs

Asymmetric acylation is a versatile strategy in synthetic organic chemistry for the preparation of small chiral compounds. Such methodology, described by Oriyama and co-workers in the desymmetrization of various *meso* diols, is based on enantioselective acylation with benzoyl chloride in the presence of chiral diamine derived from (*S*)-proline and typically proceeds with high yield and enantioselectivity.[14,15] For instance, asymmetric benzoylation of *cis* 1,2-cyclohexanediol under proline catalysis affords the enantiopure monobenzoylated product in 89% yield and 93% e.e.. We reasoned that adaptation of asymmetric acylation to our 1,3-DACHs could be a promising route to obtain monoprotected, enantiopure product. To this end, chiral diamine (**20**) was synthesized according to literature[16] and the dideoxystreptamine precursor (**17**)[17] was reacted under conditions described above. The reaction mixture was stirred overnight at -5 °C and monobenzoylated product **18** was obtained in a yield of 78% (Table 2, entry 1). Surprisingly, when the same reaction conditions were used for the DACHs protected with either a TBDMS or an allyl group no reaction was observed, even when the reaction was performed with AcCl (entry 4 and 5). Presumably also in this case acetylation was prevented by steric hindrance of the 5-*O* protective group, a presumption which is further substantiated by the unsuccessful acetylation of the 2-DOS precursors in the presence of DIPEA and AcCl (entry 6), and only slight acceleration by DMAP (entry 7). Only under the influence of Ac_2O, Et_3N and DMAP a smooth reaction was observed (entry 8), but no further enantioselective acylation was further pursued because it is clear that the accelerating potential of **20** lags that of DMAP by far.

Table 2. Acylation of 1,3-DACH



17 R = H
1 R = OTBDMS
14 R = OAll

18 R = H R_2 = Bz R_3 = H (78%)
19 R = OTBDMS R_2 = Ac R_3 = Ac (93%)

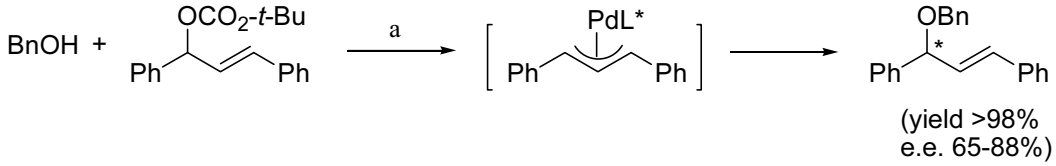
	Conditions	reactant	yield (%)
1	BzCl[a]	**17**	78 [a]
2	BzCl[a]	**1**	*no reaction*
3	BzCl[a]	**14**	*no reaction*
4	AcCl[a]	**1**	*no reaction*
5	AcCl[a]	**14**	*no reaction*
6	AcCl, DIPEA, CH_2Cl_2	**1**	*no reaction*
7	AcCl, DMAP, CH_2Cl_2	**1**	slow[b]
8	Ac_2O, Et_3N, DMAP, CH_2Cl_2	**1**	93 [c]

[a] *Reagents and conditions:* diamine **20**, BzCl or AcCl (ratio 1.5:1), 4 Å MS, EtCN, -78 °C. [b] prolonged stirring yielded (72 h) mainly starting material.[c] BORSM.

4.6 Pd-catalyzed asymmetric allylic alkylation (AAA)

Pd-catalyzed asymmetric allylic alkylation has recently been developed for the resolution of allylic alcohols *via* chiral π-allyl palladium complexes.[18,19] Grover and co-workers, for example (Scheme 4) reported an enantioselective alkylation of BnOH with a secondary carbonate in the presence of (*R*)-BINAP, with good conversion and high enantiomeric excess (65-88%).[20] We realized that adaptation of such a strategy might possibly also be applied in inverse mode for the resolution of *chiral* alcohols with *achiral* allyl carbonates, which could be directly applied in the desymmetrization of 2-DOS precursors.

Scheme 4.

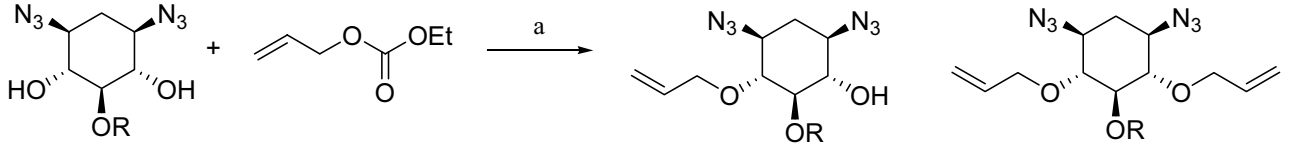


Reagents and conditions: (a) $Pd_2(dba)_3$, (*R*)-BINAP, THF, 55 °C.

Thus, in a pilot experiment TBDMS protected 1,3-DACH **1** was reacted with allyl ethyl carbonate in the presence of $Pd(dba)_2$ and 1,4-(diphenylphosphino)butane (DPPB) to give monoallylated compound **21** and diallylated compound **23** in a yield of respectively 51% and 18% (Scheme 5 and Table 3, entry 1). No reaction was observed under Ph_3P-catalysis instead of DPPB, suggesting that

the reaction is only activated by bidentate ligands. The successful monoallylation stimulated us to investigate the desymmetrization of 2-DOS precursors with several chiral phosphine ligands (Table 3).

Scheme 5.



1 R= TBDMS
13 R= Bn

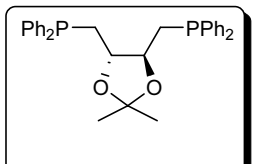
21 R= TBDMS
22 R= Bn

23 R= TBDMS
24 R= Bn

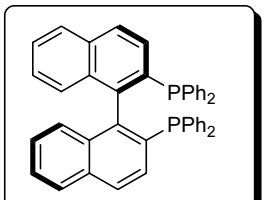
Reagents and conditions: (a) $Pd_2(dba)_3$, 1,4-(diphenylphosphino)butane, THF, 55 °C.

Firstly, under (*S*,*S*)-DIOP catalysis the monoallylated product **21** was obtained in a slightly reduced yield of 41%, accompanied by trace amounts of diallyl ether **23** (4%, entry 2). Prolonged stirring (entry 3) led to improved yields of both mono- and diallylated products but in a worse ratio. In the presence of (*R*)-BINAP only little conversion occurred (entry 4).

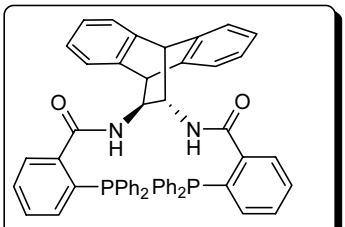
Table 3. Optimization of the Pd-catalyzed asymmetric allylic alkylation



(*S*,*S*)-DIOP (**25**)



(*R*)-BINAP (**26**)



(*S*,*S*)-anthracenyl ligand (**27**)

	diol	phosphine ligand	time (h)	temp (°C)	starting material (%)	monoallyl (%)	diallyl (%)	ee (%)
1	**1**	DPPB	1	70	-	51	18	-
2	**1**	**25**	5	20	-	41	4	-
3	**1**	**25**	24	20	-	62	26	-
4	**1**	**26**	5	20	30	25[a]	-	-
5	**13**	DPPB	2	40	10	35[a]	22[a]	-
6	**13**	**25**	2	40	30	44[a]	<5[a]	<1
7	**28**	DPPB	4	40	31	75[a]	-	-
8	**28**	**27**	4	40	40	59[a]	-	22

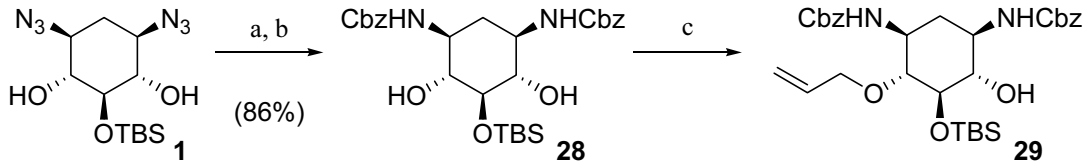
[a] BORSM.

The fact that we found only minor amounts of the diallylated compounds was taken as an indication of the enantioselectivity of the reaction. Unfortunately, it turned out rather cumbersome to define the enantiopurity of **21** with HPLC due to the lack of a UV-chromophore. For this reason the

monoallylation was repeated with benzyl-protected 2-DOS (**13**), to give under DIOP catalysis the monoallylated product (**22**) in a yield of 44%. Unfortunately, HPLC analysis on a chiral ODH column showed that the product was obtained with no enantioselectivity (<1% e.e., entry 6). Another disadvantage is that in all reactions small amounts of base-line material were observed by Staudinger reduction effected by the phosphine ligand.[21]

Most recently, a paper by Trost and co-workers showed that an asymmetric allylation with the anthracenyl ligand (**27**) gave the best yields and selectivities among four other ligands,[22] which stimulated us to investigate desymmetrization potential of ligand **27**. However, in order to avoid the undesirable Staudinger reaction, **1** was first reduced under hydrogen atmosphere, followed by protection of the amines to give Cbz-protected **28** (Scheme 6). Allylation under the optimized conditions gave no conversion at room temperature, but when the reaction was heated to 40 °C monoallylated protected 2-DOS **29** was formed in a yield of 59% with no detectable formation of the diallylated compound. To our surprise, HPLC analysis revealed that product was formed with only moderate enantioselectivity (22% e.e., entry 8) and the strategy of enantioselective allylation was further abandoned.

Scheme 6.

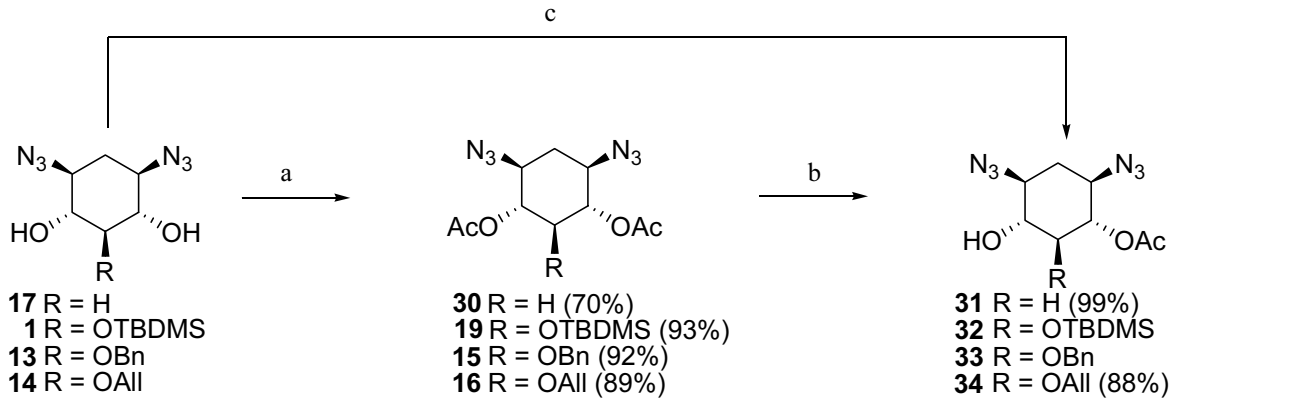


Reagents and conditions: (a) Pd/C (10%), H_2, MeOH, 3 bar, o.n.; (b) CbzCl, Et_3N, MeOH, rt, o.n.; (c) anthracenyl ligand (**27**), allyl ethyl carbonate, $Pd_2(dba)_3$, toluene, 40 °C, 3h.

4.7 Enzymatic resolutions of 1,3-DACHs

The hydrolysis or formation of the *C-O* bond of an ester, lactone or carbonate by a hydrolase is amongst the most useful enzyme-catalyzed reactions in organic synthesis.[23] Hydrolases and in particular lipases are established tools on laboratory as well as on industrial scale, primarily because of their ability to be highly active under (buffered) aqueous conditions but also in the presence of an organic cosolvent.[24] The most widely used class of hydrolases are lipases, for example pig pancreas lipase, *pseudomonas sp.* lipase, *Candida antarctica* lipase, *Candida cylindracea* lipase (CCL) and *Porcine pancreas* lipase (PPL). A further potent application of lipases lies in the resolution of alcohols, either by enantioselective hydrolysis of acetate esters or by acetylation of alcohols with an acetate donor (Scheme 7). Application of the latter potential of hydrolases for our purpose lies in the desymmetrization of *meso* 2-DOS.

Scheme 7.



17 R = H
1 R = OTBDMS
13 R = OBn
14 R = OAll

30 R = H (70%)
19 R = OTBDMS (93%)
15 R = OBn (92%)
16 R = OAll (89%)

31 R = H (99%)
32 R = OTBDMS
33 R = OBn
34 R = OAll (88%)

Reagents and conditions: (a) Ac_2O, DMAP, Et_3N, CH_2Cl_2, rt; (b) lipase, phosphate buffer, pH 6-7, 27-37 °C; (c) lipase, vinyl acetate, toluene, 27-37 °C.

Initial experiments were performed with the dideoxy analogue (**30**) with several lipases and it was found that out of 11 different enzymes (Table 5, experimental section) two were able to hydrolyze the substrate, *i.e. Candida antarctica* lipase (CAL) and *Candida cylindracea* lipase (CCL). Much to our satisfaction the monohydrolyzed product was formed in a high yield of 95% and 99%, respectively, and excellent selectivity (both >99% e.e.), which compares favorably with the reported 22% yield with Novozym 435.[25]

Unfortunately, upon application of the desymmetrization conditions to the *O*-TBDMS-protected 2-DOS precursor **19**, no hydrolysis products were obtained with any of the available enzymes (Table 5), most likely due to the fact that the (large) TBDMS group prohibits access to the enzyme's active site. The allyl and benzyl protected 2-DOS precursors were also subjected to effect hydrolysis but again neither of the enzymes were able to remove an acetyl group (Table 5).

Because it was not possible to desymmetrize the 2-DOS derivatives **15**, **16** and **19** by a commercially available enzyme, 17 esterases provided by Diversa Corporation, San Diego were screened next. Thus, the TBDMS-protected 1,3-DACH **19** was dissolved in a mixture of MeCN and phosphate buffer (pH 7.5) and the esterases were added. After incubation for 5d, reactions were filtered, solvents were evaporated and analyzed by ^{1}H NMR spectroscopy to show that again neither of the enzymes had been able to hydrolyze the starting material. In a final attempt, resolution was attempted of allyl protected 2-DOS **16** and it was rewarding to find that 6 esterases (out of 17) were indeed able to hydrolyse the substrate (Table 4), although in four cases complete deprotection occurred (entry 1,2,4,6) and under the influence of one esterase a mixture of starting material, monodeprotected product and diol was obtained (entry 3). Much to our delight, a single, monoacetylated product (**34**) was obtained (entry 5) in a yield of 88% and, more importantly, with excellent enantioselectivity (>99%.) under the action of esterase 5. The absolute stereochemistry of **34** has not yet been established.

Table 4. Desymmetrization of allyl protected DACH **16**

	enzymes	specific activity[a]	conversion (%)	ratio **14**:**34**	e.e. (%)
1	Diversa esterase 1	2.642	73	1:0	
2	Diversa esterase 2	243.422	36	1:0	
3	Diversa esterase 3	17.279	n.d.	n.d.[b]	
4	Diversa esterase 4	5.084	19	1:0	
5	Diversa esterase 5	0.032	67	0:1	>99%
6	Diversa esterase 6	8.3	100	1:0	

[a] activity: units/mg protein, 1 unit=1 μmol/min of pNP released from 5 mM *p*-NP-butyrate, pH 7.5, 37 °C [b] three products **14**, **16** and **34** ratio could not be determined.

4.8 Concluding remarks

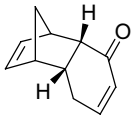
In the presence of an enantiopure bidentate phosphine ligand, Pd-catalyzed 1,4-hydrogen migration of the previously prepared *meso* diol-**3** successfully led to enantiopure ketone (+)-**6** with high enantiomeric excess, although the yield was suppressed by the formation of a hitherto unidentified side-product. Sharpless asymmetric dihydroxylation of a late-stage intermediate of the route presented in chapter 3 yielded the desired diol in high yield (96%) but with moderate enantiomeric excess. A new route toward 2-DOS precursors was developed from kanamycin A, to give in three steps a benzyl or allyl protected *meso* 2-DOS analogue in overall yields of 39% and 40%, respectively. Desymmetrization of the TBDMS, benzyl and allyl diazidocyclitols by asymmetric acylation was not successful due to low reactivity of the hydroxyls. A Cbz-protected 2-DOS derivative could be desymmetrized by asymmetric allylic alkylation in reasonable yield but the enantiomeric excess was low (22%). Finally, enzymatic resolution of a dideoxystreptamine precursor was successfully achieved in high yield 99% and with excellent enantiomeric excess (99%), under the action of *Candida Cylindracea* lipase. The latter enzyme, however, turned out inactive for protected 2-DOS analogues, and only a single esterase obtained from Diversa was able to desymmetrize an allyl protected diazido cyclitol, to give the desired product in a most gratifying yield of 88% and with excellent enantiomeric excess (>99%). The latter product shows promise as an orthogonally protected building scaffold for the development of enantiopure, novel 2-DOS based RNA-ligands.

4.9 Acknowledgements

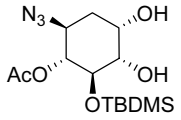
S. Groothuys is acknowledged for his work on the enantioselective Pd-catalyzed 1,4-hydrogen migration reaction. B. Verheijen has made a significant experimental contribution to this chapter. Prof. Dr. H. E. Schoemaker and Dr. N. Sereinig (DSM, Geleen) are gratefully acknowledged for

their hospitality and help in optimization of conditions for the enzymatic resolutions. We are grateful to Dr. D. P. Weiner (Diversa Corporation, San Diego) for the kind provision of 17 esterases. Dr. P. Botman (Chiralix B.V., Nijmegen) is kindly acknowledged for the suggestion to desymmetrize 2-DOS with the asymmetric allylic alkylation method. Dr. I. C. Lennon (Dow, Cambridge) kindly provided a sample of the (*S*,*S*)-anthracenyl ligand. K. Koch, R. van den Berg and C. Schortinghuis were very helpful in the HPLC measurements.

4.10 Experimental Section



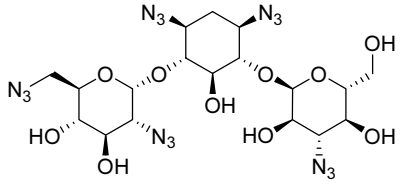
(+)-(1*R*,2*R*,7*R*,8*S*)-Tricyclo[6.2.1.0^{2,7}]undeca-4,9-dien-3-one (6)
To a solution of **3** (50 mg, 0.28 mmol) in MeCN (3 mL), was added (*S,S*)-Trost ligand **7** (12 mg, 0.017 mmol, 6 mol%), and optionally HCO_2NH_4 (27 mg, 0.43 mmol). Under an argon atmosphere 5 mol% palladium complex was added. The reaction was stirred for given time at 60 °C or reflux. Spectral data are in agreement with the data shown in chapter 3. The reaction was monitored by GC. For entry 1 of the crude mixture was purified by column chromatography (EtOAc/*n*-heptane, 1/5), and **4** was obtained with an optical purity of 97% ee by HPLC (chiralpak AD, elution with *n*-hexane/IPA 99/1, flow: 1 mL/min); retention times (min): 14.00 and 18.45.



(+ or -)-(1*R*,2R,3*S*,4*S*,5*R*)-5-azido-3-[(*tert*-butyldimethylsilyl)oxy]cyclohexane-4-acetyloxy-1,2-diol (9)
A 25-mL round-bottomed flask, equipped with a magnetic stirrer, was charged with 5 mL of *t*-BuOH, 5 mL of water, and 1.4 g of AD-mix-α or AD-mix-β.[6] Stirring at room temperature produced two clear phases; the lower aqueous phase appears bright yellow. Methanesulfonamide (95 mg, 1 equiv based on 1 mmol of olefin) was added at this point. The mixture was cooled to 0 °C whereupon some of the dissolved salts precipitated. One mmol of olefin was added at once, and the heterogeneous slurry was stirred vigorously at 0 °C for 24 h (progress was monitored by TLC). While the mixture was stirred at 0 °C, solid sodium sulfite (1.5 g) was added and the mixture was allowed to warm to room temperature and stirred for 30-60 min. EtOAc (10 mL) was added to the reaction mixture, and after separation of the layers, the aqueous phase was further extracted with the organic solvent (3 X 5 mL) and the combined organic layers were washed with 2 N KOH). The combined organic extracts were dried with Na_2SO_4 and concentrated to give the diol and the ligand. This crude product was purified by flash chromatography (EtOAc/hexanes; 1/5 the ligand does not move in this solvent system) to afford the 1,2-diol. Spectroscopic data were in agreement with the data obtained in chapter 3. An optical purity of 21% ee by HPLC (chiralpak AD-H, elution with *n*-hexane/IPA 98/2, flow: 1 mL/min); retention times (min): 17.19 and 22.26.

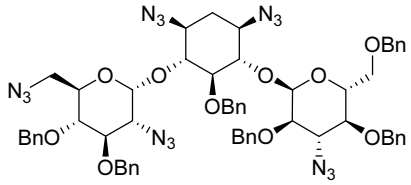
General procedure for the synthesis of the triflylazide solution in CH_2Cl_2 (0.6 M)
A solution of NaN_3 (8.19 g, 126 mmol) in H_2O (20 mL), was cooled to 0 °C and CH_2Cl_2 was added (20 mL). Tf_2O (11 mL, 63 mmol) was added slowly to the vigorously stirring solution, and the reaction mixture was stirred for 2 hours at 0 °C after which it was quenched carefully with $NaHCO_3$ (sat. aq.) until CO_2 evolution ceases. The organic phase was collected and the aqueous phase was washed with CH_2Cl_2 (2x 15 mL) the combined organic phases were washed with sat. $NaHCO_3$. To give a solution of approximately 0.6 M triflyl azide in CH_2Cl_2 (50 mL).[13]

General procedure for the acetylation
Protected aminocyclitol was dissolved in CH_2Cl_2 and Et_3N (4 equiv) and Ac_2O (4 equiv) and DMAP (cat) were added to the reaction. The reaction was stirred for 4 hours, diluted with CH_2Cl_2, washed with brine and dried with Na_2SO_4, followed by evaporation of the organic solvent and purified by flash column chromatography.



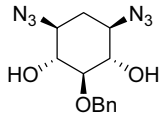
3,4',6',6''-Tetrazido-kanamycin A (10)

Kanamycin-A sulfate (3.86 g, 6.62 mmol) and $ZnCl_2$(cat.) were dissolved in H_2O (80 mL). Et_3N (11.0 mL, 79.2 mmol) and, carefully, MeOH (266 mL). Triflylazide in CH_2Cl_2 (0.6 M, 80 mL) was added to the vigorously stirring solution at once, the solution becomes colorless. After stirring for three hours the solvents were carefully evaporated and the product was purified by flash chromatography (EtOAc/MeOH, 9/1), to give **10** as a colorless oil (2.07 g, 75%). R_f 0.4 (MeOH/EtOAc, 1/9). IR υ_{max} film: (cm^{-1}) 2473, 2359, 2114, 1384, 1234, 1188, 1150, 1030, 953, 612, 565, 494. ^{1}H NMR (MeOD, 400 MHz, ppm): δ 5.27 (d, J = 3.8 Hz, 1H), 5.22 (d, J = 3.8 Hz, 1H), 4.19-3.98 (m, 2H), 3.82-3.33 (m, 15H), 2.38 (dt, J = 4.0, 12.9 Hz, 1H), 1.56 (dt, J = 12.4, 12.4 Hz, 1H). ^{13}C NMR (MeOD, 75 MHz, ppm): δ 102.2, 99.6, 84.8, 81.6, 75.6, 74.7, 73.7, 73.3, 73.2, 72.2, 71.9, 69.7, 68.4, 62.1, 61.9, 60.8, 52.7, 33.4. HRMS (FAB) *m/z* calcd for $C_{18}H_{29}O_{11}N_{12}$ $(M+H)^+$: 589.2079, found 589.2088.



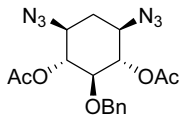
Tetrazido-hepta-*O*-benzyl-kanamycin A (11)

Compound **10** (502 mg, 0.85 mmol) was dissolved in DMF. TBAI (0.2 g, cat) was added and the reaction was cooled to 0 °C. NaH (0.400 g, 5.76 mmol) and BnBr (2.0 mL, 16 mmol) were added. The reaction was stirred for 4h at rt. The reaction was quenched with NH_4Cl (sat) and extracted with *i*-PrOH/EtOAc (1/9, 10 mL). Wash three times with water and evaporate the organic solvent. Flash column chromatography (EtOAc/*n*-heptane, 1/5), to yield compound **11** as a white foam (75%). $[\alpha]_D^{20}$ +97.4 (c 0.42; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 3029, 2902, 2100, 1743, 1495, 1452, 1361, 1259, 1068, 1028, 910, 736, 697, 580. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.45-6.81 (m, 35H), 5.56 (d, J = 3.7 Hz, 2H), 5.04 (d, J = 12.0 Hz, 1H), 4.89-4.47 (m, 9H), 4.42 (d, J = 12.0 Hz, 1H), 4.30 (ddd, J = 2.4, 4.6, 9.9 Hz, 1H), 4.21-4.09 (m, 3H), 4.16 (t, J = 18.8 Hz, 1H), 3.77-3.22 (m, 14H), 3.11 (dd, J = 1.8, 11.0 Hz, 1H), 2.84 (dd, J = 2.4, 11.0 Hz, 1H), 2.39 (td, J = 4.4, 13.1 Hz, 1H), 1.65 (dd, J = 12.4, 25.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 138.2-137.2, 128.4-127.1, 125.6, 97.2, 96.0, 82.6, 81.8, 79.3, 78.4, 76.8, 76.7, 76.6, 76.0, 75.6, 75.1, 74.5, 74.5, 74.1, 73.6, 73.2, 70.9, 70.0, 67.7, 65.5, 60.5, 59.5, 51.4, 32.4. HRMS (FAB) *m/z* calcd for $C_{67}H_{71}O_{11}N_{12}$ $(M+H)^+$: 1219.5365, found: 1219.5366.



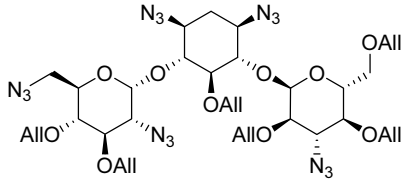
(1*R*,2*r*,3*S*,4*R*,6*S*)-4,6-Diazido-2-*O*-benzyl-cyclohexane-1,3-diol (13)

AcCl (1.5 mL) was added to MeOH (15 mL) at 0 °C. The obtained 1 N HCl/MeOH was added to **11** (200 mg, 0.24 mmol) and the reaction mixture was refluxed over night. The reaction was quenched with $NaHCO_3$ (sat.) and extracted with EtOAc. Flash column chromatography (EtOAc/*n*-heptane, 1/3), to yield **7** as a white solid (31 mg, 70%). R_f 0.11 (EtOAc/*n*-heptane, 1/3). Mp 130.9 °C. IR υ_{max} film: (cm^{-1}) 3430, 2359, 2105, 1454, 13565, 1127, 1025, 744, 700. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.49-7.28 (m, 5H), 4.83 (s, 2H), 3.51-3.31 (m, 4H), 3.24 (t, J = 7.8 Hz, 1H), 2.19 (dt, J = 13.2, 3.9 Hz, 1H), 1.27 (dt, J = 13.2, 12.9 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): 128.8, 128.0, 83.4, 75.7, 60.5, 32.1. HRMS (EI) *m/z* calcd for $C_{13}H_{16}O_3N_6$ $(M+H)^+$: 304.1284, found: 304.1294.



(1*R*,2*r*,3*S*,4*R*,6*S*)-1,3-Di-*O*-acetyl-4,6-diazido-2-*O*-benzyl-cyclohexane (15)

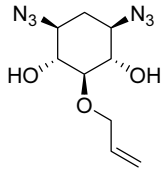
Acetylation of **13** (45 mg, 0.15 mmol) *via* general procedure and purification by flash column chromatography (EtOAc/*n*-heptane, 1/3) to yield **15** (53 mg, 92%). R_f 0.53 (EtOAc/*n*-heptane, 1/1). IR υ_{max} film: (cm^{-1}) 2099, 1745, 1564, 1453, 1372, 1218, 1065, 1034, 897, 862, 746, 699. ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.48-7.18 (m, 5H), 5.06 (t, J = 9.9 Hz, 2H), 4.60 (s, 2H), 3.52-3.39 (m, 3H), 2.29 (dt, J = 13.5, 4.1 Hz, 1H), 2.02 (s, 6H), 1.57 (dt, J = 13.0, 12.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): 169.2, 128.4, 127.6, 79.6, 74.9, 58.6, 32.1, 21.0. HRMS (EI) *m/z* calcd for $C_{17}H_{20}O_5N_6$ $(M+Na)^+$: 411.



Tetrazido-hepta-*O*-allylkanamycin A (12)

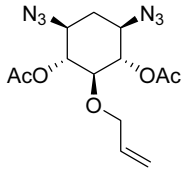
Compound **10** (517 mg, 0.88 mmol) was dissolved in DMF, TBAI (0.2 g, cat.) was added, after which the reaction was cooled to 0 °C. NaH (545 mg, 7.85 mmol) and allylbromide (1.0 mL, 12 mmol) were added. The reaction mixture was stirred for 2 h at rt. Quenched with NH_4Cl and

extracted with 2-propanol/EtOAc (1/9, 10 mL) and washed three times with water, dried with Na_2SO_4 and evaporated. Flash column chromatography (EtOAc/*n*-heptane, 1/5) to yield **12** as yellow oil (596 mg, 78%). R_f 0.35 (EtOAc/*n*-heptane, 1/3). IR υ_{max} film: (cm^{-1}) 2871, 2360, 2339, 2250, 2103, 1351, 1262, 1075, 1031, 996, 913, 734, 668, 646, 412. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.90-5.78 (m, 7H), 5.55 (d, *J* = 3.7 Hz, 1H), 5.47 (d, *J* = 3.8 Hz, 1H), 5.31-5.12 (m, 14H), 4.45 (d, *J* = 10.0 Hz, 1H), 4.37-3.84 (m, 14H), 3.85-3.62 (m, 2H), 3.58-3.22 (m, 14H), 2.35 (d, *J* = 8.0 Hz, 1H), 1.21 (t, *J* = 4.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 134.9, 134.6, 134.5, 134.4, 134.3, 134.0, 133.9, 118.6, 117.7, 117.0, 116.5, 116.5, 115.7, 97.3, 96.0, 82.6, 81.6, 79.0, 78.2, 75.9, 74.3, 74.2, 74.0, 73.6, 72.6, 72.6, 70.7, 69.9, 68.3, 65.2, 61.0, 59.3, 51.6, 32.7.



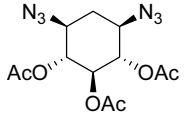
(1*R*,2*r*,3*S*,4*R*,6*S*)-4,6-Diazido-2-*O*-allyl-cyclohexane-1,3-diol (14)

AcCl (1.5 mL) was added to MeOH (15 mL) at 0 °C. The obtained 1 N HCl/MeOH was added to **12** (200 mg, 0.23 mmol) and the reaction was refluxed for 16 hours. Quench with $NaHCO_3$ (sat.) and extract with EtOAc. Wash with water and dry with $MgSO_4$ and evaporate the solvent. Purify by flash column chromatography (EtOAc/*n*-heptane, 1/3), to obtain **14** as a white solid (58 mg, 68%). R_f 0.11 (EtOAc/*n*-heptane, 1/3). IR υ_{max} film: (cm^{-1}) 3408, 2920, 2100, 1732, 1451, 1348, 1260, 1070, 1028, 925, 571, 443, 424. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.01-5.86 (m, 1H), 5.23-5.11 (m, 4H), 4.18 (dt, *J* = 1.4, 5.6 Hz, 2H), 3.50-3.38 (m, 3H), 2.20 (dt, *J* = 4.6, 13.3 Hz, 1H), 1.38 (dt, *J* = 12.6, 13.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 134.5, 117.8, 83.2, 76.2, 74.5, 60.5, 32.2. HRMS (CI) *m/z* calcd for $C_9H_{14}O_3N_6$ $(M+)^+$: 254.1128, found: 254.1116.



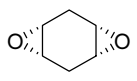
(1*R*,2*r*,3*S*,4*R*,6*S*)-1,3-Di-*O*-acetyl-2-*O*-allyl-4,6-diazido-cyclohexane (16)

Acetylation of **14** (30 mg, 0.12 mmol) *via* general procedure and purification by flash column chromatography (EtOAc/*n*-heptane, 1/3) to yield **16** (36 mg, 89%) as a colorless oil. R_f 0.53 (EtOAc/*n*-heptane, 1/1). IR υ_{max} film: (cm^{-1}) 2920, 2098, 1744, 1557, 1373, 1219, 1152, 1064, 1034, 926, 615, 447. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.75 (ddt, *J* = 17.3, 10.4, 5.7 Hz, 1H), 5.32-5.11 (m, 2H), 5.01 (t, *J* = 9.9 Hz, 2H), 4.07 (dt, *J* = 1.4, 5.6 Hz, 2H), 3.45 (m, 3H), 2.28 (dt, *J* = 13.3, 4.6 Hz, 1H), 2.13 (s, 6H), 1.54 (dt, *J* = 13.2, 12.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 169.5, 134.0, 117.2, 79.1, 74.8, 73.8, 58.2, 31.8, 20.8. HRMS (CI) *m/z* calcd for $C_{13}H_{18}O5N_6$ $(M+H)^+$: 339.1417, found: 339.1428.



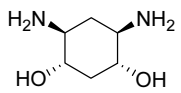
(1*R*,2*r*,3*S*,4*R*,6*S*)-1,2,3-Tri-*O*-acetyl-4,6-diazidocyclohexane

Kanamycin derivative (**10**) (100 mg, 0.17 mmol) was dissolved in an 1 N HCl/MeOH solution (1.4 mL). The reaction mixture was refluxed overnight and quenched with saturated $NaHCO_3$. The solvents were evaporated and the crude reaction mixture was purified by flash column chromatography (EtOAc/*n*-heptane, 1/1), to obtain the triol as an oil. The oil was dissolved in Ac_2O (0.5 mL, 5 mmol) and one drop of $BF_3.Et_2O$ was added to reaction mixture. The reaction mixture was stirred 10 min and quenched with water and extracted with EtOAc, dry with Na_2SO_4 and evaporate the solvents. Purify by flash column chromatography (EtOAc/*n*-heptane, 1/3) to obtain a colorless oil (10 mg, 20%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.09-4.81 (m, 3H), 3.61-3.55 (m, 2H), 2.33 (dt, *J* = 4.6, 13.6 Hz, 1H), 2.04 (s, 6H), 2.01 (s, 3H), 1.58 (q, *J* = 12.8 Hz, 1H); in agreement with literature.[26]



(1*R*,2*S*,4*R*,5*S*)-1,2:4,5-Diepoxycyclohexane

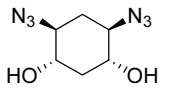
To a cold suspension (0 °C) of *m*-CPBA (77% pure, 18 g, 75 mmol) in CH_2Cl_2 (150 mL) was added a solution of 1,4-cyclohexadiene (3.6 mL, 37 mmol) in CH_2Cl_2 (50 mL). This mixture was stirred at 0 °C for 8 hours and at room temperature for 16 hours. The mixture was filtrated and washed with phosphate buffer (pH 7.0). Flash chromatography (toluene/acetone, 7/1) to yield the compound as a white solid. R_f 0.06 (EtOAc/*n*-heptane, 1/10). IR υ_{max} film: (cm^{-1}) 2991, 2906, 1717, 1470, 1418, 1354, 1263, 1022, 834. ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 3.07 (s, 4H), 2.74 (d, *J* = 22.4 Hz, 2H), 2.25 (d, *J* = 22.4 Hz, 2H); in agreement with literature.[27]



(1*R*,3*S*,4*S*,6*R*)-1,3-Diamino-cyclohexane-4,6-diol

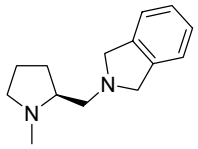
The diepoxide (300 mg, 2.7 mmol) was dissolved in benzylamine (1.17 mL, 10.7 mmol) under inert atmosphere. The reaction was stirred at 150 °C for 8 hours. Et_2O (3 mL), was added and the reaction was cooled to -20 °C, filter off and rinse with cold Et_2O to yield the compound (730 mg, 80%) as a slightly yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.32 (br s, 10H),

3.94 (d, *J* = 12.9 Hz, 2H), 3.80 (d, *J* = 12.9 Hz, 2H), 3.31 (ddd, *J* = 11.3, 9.2, 4.3 Hz, 2H), 2.50 (dt, *J* = 12.4, 3.8 Hz, 1H), 2.41-2.21 (m, 3H), 1.38 (dt, *J* = 11.7, 11.6 Hz, 1H), 0.83 (dt, *J* = 11.8, 11.7 Hz, 1H); in agreement with literature.[27] A suspension of Pd/C (10%, 243 mg, 0.23 mmol) and $Pd(OH)_2$(C) (20%, 322 mg, 0.46 mmol) in MeOH (5 mL) was added to a solution of the benzylaminodideoxystreptamine analogue (155 mg, 0.46 mmol) in MeOH/AcOH (3/1, 8 mL). Stir for 48 hours under H_2 atmosphere, filter off over Celite® and evaporate the solvent to yield compound as a white solid.



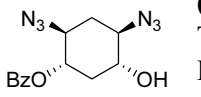
(1*R*,3*S*,4*S*,6*R*)-1,3-Diazido-cyclohexane-4,6-diol (17)

Dideoxystreptamine (146 mg, 0.46 mmol) and $ZnCl_2$ (cat.) were dissolved in water (3 mL). Et_3N (0.418 mL), MeOH (10 mL, slowly) and 0.6 M triflylazide solution (3 mL) in CH_2Cl_2 were added. The reaction was stirred vigorously for three hours. The solvents were evaporated carefully and flash chromatography (EtOAc/*n*-heptane, 3/1) to yield compound **17** as a white solid (40 mg, 53% over two steps). R_f 0.6 (EtOAc). mp 70.7 °C. IR υ_{max} film: (cm^{-1}) 3369, 2921, 2358, 2100, 1737, 1597, 1452, 1370, 1257, 1234, 1072, 1028, 585, 438. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.46 (ddd, *J* = 11.4, 9.2, 4.4 Hz, 2H), 3.29 (ddd, *J* = 11.4, 8.4, 3.6 Hz, 2H), 2.37-2.11 (m, 2H), 1.48 (dt, *J* = 12.9, 11.6 Hz, 1H), 1.31 (dt, *J* = 13.2, 12.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 71.2, 64.2, 38.4, 31.8. MS (CI) m/z (M+H+): 199. HRMS (EI) *m/z* calcd for $C_{16}H_{29}O_5SiN_6$ $(M)^+$: 198.0865, found: 198.0866.



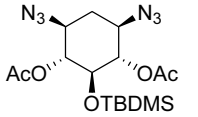
(*S*)-2-(Isoindolinylmethyl)-*N*-methylpyrrolidine (20)

The chiral diamine was prepared according to literature.[16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.25 (m, 4H), 3.96 (s, 4H), 3.07 (td, *J* = 9.1, 1.9 Hz, 1H), 2.93 (dd, *J* = 11.9, 4.6 Hz, 1H), 2.65 (dd, *J* = 11.8, 8.1 Hz, 1H), 2.44 (s, 3H), 2.38-2.29 (m, 1H), 2.25-2.16 (m, 1H), 2.12-1.98 (m, 1H), 1.95-1.59 (m, 3H); in agreement with literature.[16]



(-)or(+)(1*R*,2*R*,4*S*,5*S*)-2,4-Diazido-5-benzoyloxy-cyclohexan-1-ol (18)

To molecular sieves 4Å was added dideoxystreptamine analogue **17** (10 mg, 0.05 mmol) in EtCN and the reaction mixture was cooled to -78 °C. Benzoyl chloride (9 µL, 0.05 mmol) and chiral diamine (11 mg, 0.075 mmol) were now added and the reaction mixture was stirred for 24 h. (The molecular ratio of the diol: benzoyl chloride :diamine has to be 1:1.5:1.).The reaction mixture was now quenched with a phosphate buffer (pH 7) and the organic materials were extracted with Et_2O and the combined extracts were dried with Na_2SO_4. Evaporate the solvent and purify by flash column chromatography (EtOAc/*n*-heptane, 1/5) to yield **18** (7 mg, 47%, 78% BORSM). IR υ_{max} film: (cm^{-1}) 2102, 1719, 1269, 1070, 713, 668. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.19-8.01 (m, 2H), 7.66-7.61 (m, 1H), 7.50-7.34 (m, 2H), 5.07-4.88 (m, 1H), 3.79-3.59 (m, 2H), 3.43-3.33 (m, 1H), 2.58 (dt, *J* = 12.8, 4.9 Hz, 1H), 2.38 (dt, *J* = 12.8, 4.9 Hz, 1H), 1.45 (m, 2H).

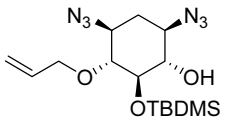


(1*R*,2*S*,3*r*,4*R*,5*S*)-2,4-(diacetoxy)-1,5-diazido-3-[(*tert*-butyldimethylsilyl)oxy] cyclohexane (19)

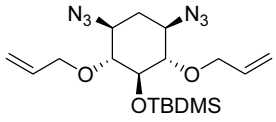
Compound **1** (100 mg, 0.30 mmol) was dissolved in CH_2Cl_2 (3 mL) and Et_3N (84 µL, 0.75 mmol), Ac_2O (72 µL, 0.75 mmol) and DMAP (cat) were added. The reaction mixture was stirred for 2 hours, diluted with CH_2Cl_2, washed with brine and dried with Na_2SO_4. Evaporation of the organic solvent and purification by flash column chromatography (EtOAc/*n*-heptane, 1/10 to 1/5) to yield **19** as colorless oil (117 mg, 93%). IR υ_{max} film: (cm^{-1}) 2951, 2930, 2857, 2098, 1746, 1372, 1250, 1215, 1144, 1096, 1067, 1032, 979, 896, 878, 836, 779. ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 4.97 (t, *J* = 9.6 Hz, 2H), 3.69 (t, *J* = 9.5 Hz, 1H), 3.40 (dt, *J* = 11.4, 4.5 Hz, 2H), 2.30 (dt, *J* = 10.0, 3.3 Hz, 1H), 2.17 (s, 6H), 1.62 (dt, *J* = 12.9, 12.8 Hz, 1H), 0.85 (s, 9H), 0.08 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 169.6, 77.4, 77.1, 76.8, 75.7, 72.2, 58.7, 31.8, 25.5, 22.8, 21.3, 17.8, -4.3. HRMS (EI) *m/z* calcd for $C_{16}H_{29}O_5SiN_6$ $(M+H)^+$: 413.1969, found: 413.1971.

General procedure for AAA
To a degassed mixture of $Pd_2(dba)_3$ (4 mol%) and a chiral phosphine ligand or DPPB (8 mol%) in a given solvent was added allylethylcarbonate (1.5 equiv) and 2-DOS. After 10 min a yellow solution was obtained. The reaction mixture was stirred under an argon atmosphere for 30 min to 4h and the volatiles were removed under reduced pressure and the residue was purified by flash column chromatography directly to afford the allyl protected compound.

(±)-(1*S*,2*R*,3*R*,4*S*,5*R*)-3-(allyloxy)-4,6-diazido-2-[(*tert*-butyldimethylsilyl)oxy]cyclohexan-1-ol (21) and (1*R*,2*S*,3*r*,4*R*,5*S*)-(1*R*,2*S*,3*r*,4*R*,5*S*)-2,4-(diallyloxy)-1,5-diazido-3-[(*tert*-butyldimethylsilyl)oxy] cyclohexane (23)
Allylation of **1** (20 mg, 0.06 mmol) *via* general procedure or AAA:
With DPPB (8 mol%, 4 mg), purification by flash column chromatography (EtOAc/*n*-heptane, 1/10) to yield **21** (23 mg, 51%) and **23** (9 mg, 18%).
With BINAP (8 mol%, 2 mg), purification by flash column chromatography (EtOAc/*n*-heptane, 1/10) to yield **21** (4 mg, 25%) and starting material **1** (6 mg, 30%).
With DIOP (8 mol%, 2 mg), purification by flash column chromatography (EtOAc/*n*-heptane, 1/10) to yield **24** (9 mg, 41%) and **23** (1 mg, <4%).

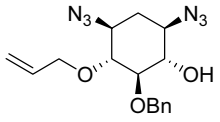


21: R_f 0.46 (EtOAc/*n*-heptane, 1/5). IR υ_{max} film: (cm^{-1}) 2948, 2100, 1453, 1074, 752, 699. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.09-5.88 (m, 1H), 5.39-5.18 (m, 2H), 4.42-4.21 (m, 2H), 3.45-3.18 (m, 4H), 3.17-2.96 (m, 1H), 2.32-2.08 (m, 1H), 1.40-1.19 (m, 1H), 0.91 (s, 9H), 0.27 (s, 3H), 0.12 (s, 3H). HRMS (CI) *m/z* calcd for $C_{15}H_{29}O_3SiN_6$ $(M+H)^+$: 369.2071, found: 369.2077.

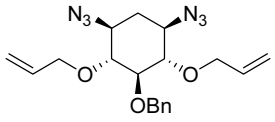


23: R_f 0.66 (EtOAc/*n*-heptane, 1/5). IR υ_{max} film: (cm^{-1}) 2930, 2101, 1257, 1130, 1080, 836, 779, 668. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.09-5.88 (m, 2H, allyl), 5.32-5.12 (m, 4H, Allyl), 4.40-4.19 (m, 4H, CH), 3.55-2.82 (m, 5H, CH), 2.22-2.05 (m, 1H, CH_2a), 1.30-1.19. (m, 1H, CH_2b), 0.93 (s, 9H, *t*-Bu), 0.87 (s, 3H, MeSi), 0.12 (s, 3H, MeSi). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 134.7, 134.2, 117.6, 116.6, 84.4, 83.7, 64.2, 60.3, 32.8, 26.3, 18.4, -3.5, -4.3. HRMS (CI) *m/z* calcd for $C_{18}H_{33}O_3SiN_6$ $(M+H)^+$: 409.2386, found:409.2384.

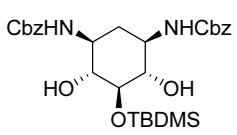
(1*S*,2*R*,3*R*,4*S*,6*R*)-3-(allyloxy)-4,6-diazido-2-(benzyloxy)cyclohexan-1-ol (22) and (1*R*,2*S*,3*r*,4*R*,5*S*)-2,4-(diallyloxy)-1,5-diazido-3-(benzyloxy)cyclohexane (24)
With DPPB (8 mol%, 4 mg), purification by flash column chromatography (EtOAc/*n*-heptane, 1/10) to yield starting material **5** (2 mg, 10%), **22** (7 mg, 35%) and **24** (5 mg, 22%).
With DIOP (8 mol%, 2 mg), purification by flash column chromatography (EtOAc/*n*-heptane, 1/10) to yield starting material **5** (6 mg, 30%), **22** (7 mg, 44%) and **24** (<1 mg, <5%).



22*: R_f* 0.44 (EtOAc/*n*-heptane, 2/3). IR υ_{max} film: (cm^{-1}) 2100, 1453, 1362, 1257, 1070,699. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.49-7.21 (m, 5H), 6.12-5.89 (m, 1H), 6.00 (dd, *J* = 5.7, 11.6 Hz, 2H), 4.95 (d, *J* = 11.3 Hz, 1H), 4.72 (d, *J* = 11.1 Hz, 1H), 4.31 (br s, 2H), 3.46-3.19 (m, 5H), 2.22-2.01 (m, 1H), 1.68-1.48 (m, 1H). HPLC optical purity of 0.05% (ADH-H, 1.0 mL/min, iPrOH/hexane, 3/97, 20 µL): retention times (min): 24.141 and 28.120, the enantiomeric excess was 0.8%.



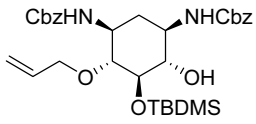
24*: R_f* 0.65 EtOAc/*n*-heptane, 2/3) IR υ_{max} film: (cm^{-1}) 2100, 1457, 1259, 1077, 700. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.45-7.30 (m, 5H), 6.18-5.89 (m, 2H), 5.30-18 (m, 4H), 4.36 (br s, 2H), 4.41-4.31 (m, 2H), 4.30-21 (m, 2H), 3.49-3.31 (m, 3H), 3.20 (t, *J* = 9.6 Hz, 2H), 2.21-2.01 (m, 1H), 1.67-1.49 (m, 1H).^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 128.3, 127.6, 82.9, 74.9, 67.2, 66.6, 57.7, 54.7, 30.0, 1.5. HRMS (CI) *m/z* calcd for $C_{19}H_{25}O_3N_6$ (M+H): 385.



(1*R*,2*r*,3*S*,4*R*,6*S*)-4,6-[di(benzyloxycarbonyl)amino]-2-[(*tert*-butyldimethylsilyl) oxy]cyclohexane-1,3-diol (28)

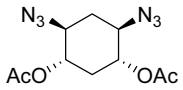
To a solution of 4,6-diazidocyclohexanetriol **1** (20 mg, 0.093 mmol) in MeOH was added Pd/C (spatula). After the mixture had been stirred for 14 h under 3 bar of H_2, Pd/C was filtered off and the filtrate was concentrated to yield the product (14 mg, 95%). IR υ_{max} film: (cm^{-1}) 3367, 1471, 1121, 836.7, 780, 611.

The crude compound (92 mg, 0.095 mmol) was now dissolved in MeOH (3 mL) and cooled to 0 °C. CbzCl (97 µL, 0.32 mmol) and Et_3N (95 µL, mmol) were added and the reaction mixture was stirred overnight. The crude product was dried (Na_2SO_4), concentrated under reduced pressure, and purified by flash chromatography (EtOAc/*n*-heptane, 1/5) to give (160 mg, 91%) of compound **28** as a white solid. IR υ_{max} film: (cm^{-1}) 2925, 1688, 1548, 905, 729. ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 7.42-7.21 (m,10H, arom), 5.12-4.94 (m, 4H, CH_2), 3.62-3.45 (m, 2H, CH), 4.42-3.25 (m, 1H, CH), 3.25-3.14 (m, 2H, CH), 2.09-1.89 (m, 1H, CH_2a), 1.49-1.3 (m, 1H, CH_2b), 0.86 (s, 9H, *t*-Bu), 0.12 (s, 3H, MeSi), 0.06 (s, 3H, MeSi). ^{13}C NMR (CD_3OD, 100 MHz, ppm): δ 158.6, 158.2, 138.3, 129.4, 128.8, 78.6, 77.7, 76.5, 67.4, 53.4, 26.2, 19.2, -3.5, -4.5. HRMS (CI) *m/z* calcd for $C_{28}H_{41}O_7SiN_2$ $(M+H)^+$: 545.2683, found: 545.2669.



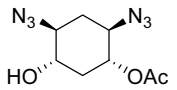
(+ or -)-(1*S*,2*R*,3*R*,4*S*,6*R*)-3-(allyloxy)-4,6-[di(benzyloxycarbonyl)amino]-2-[(*tert*-butyldimethylsilyl)oxy]-cyclohexanan-1-ol (29)

Using general procedure **AAA**. R_f 0.6 (EtOAc/*n*-heptane, 1/1). IR υ_{max} film: (cm^{-1}). 2969, 1378, 1303, 1160, 1128, 950, 816. ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 7.41-7.21 (m, 10H), 5.91-5.62 (m, 1H), 5.21-4.77 (m, 4H), 4.42-3.25 (m, 3H), 4.12-3.89 (m, 1H), 3.58-3.36 (m, 2H), 3.12-2.85 (m, 1H), 1.99-1.72 (m, 1H), 1.45-1.38 (m, 1H), 0.81 (s, 9H), 0.07 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ.155.8, 136.6, 134.9, 128.7, 128.6, 117.6, 73.3, 67.0, 52.4, 51.1, 26.0, 18.4, 1.2, -3.8, -4.5. HRMS (CI) *m/z* calcd for $C_{31}H_{44}O_7SiN_2$ $(M+H)^+$: 585.2996, found: 585.2990. HPLC optical purity of 21% ee (OD-H, 1.0 mL/min, iPrOH/hexane, 10/90, 5 µL): retention times (min): 43.45 and 52.20.



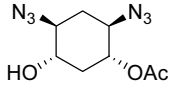
(1*R*,3*S*,4*S*,6*R*)-1,3-Diazido-4,6-di-*O*-acetyl-cyclohexane (30)

Compound **17** (40 mg, 0.20 mmol) was dissolved in CH_2Cl_2 (5 mL). Et_3N (30 µL, 0.42 mmol), Ac_2O (20 µL, 0.42 mmol) and DMAP (cat) were added to the reaction mixture. Stir for three hours. Evaporate the solvents and flash chromatography (EtOAc/*n*-heptane, 2/3) to yield **30** (40 mg, 70%) as a colorless oil. R_f 0.8 (EtOAc/*n*-heptane, 2/1). ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 4.77 (ddd, *J* = 11.7, 9.9, 4.8 Hz, 2H), 3.51 (ddd, *J* = 12.3, 9.6, 4.5 Hz, 2H), 2.46 (dt, *J* = 12.6, 4.6 Hz, 1H), 2.26 (dt, *J* = 13.5, 4.5 Hz, 1H), 2.09 (s, 6H), 1.49 (dt, *J* = 12.3, 11.7 Hz, 1H), 1.41 (dt, *J* = 13.5, 12.6 Hz, 1H); in agreement with literature.[13]



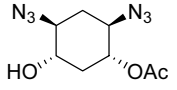
(±)-(1*R*,3*S*,4*S*,6*R*)-1,3-Diazido-4-*O*-acetyl-cyclohexan-6-ol (31)

Compound **30** (10 mg, 0.036 mmol) was dissolved in THF (1 mL) and cooled to 0 °C. LiOH (0.85 mg, 0.036 mmol) was dissolved in water (100 µL) and added slowly to the substrate. Stir for three hours at 0 °C. Evaporate the solvent and purify by flash column chromatography (EtOAc/*n*-heptane, 1/4) to yield **31** (2.7 mg, 32%, 92% BORSM). R_f 0.25 (EtOAc/*n*-heptane, 1/3). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.73 (ddd, *J* = 4.5, 9.8, 11.7 Hz, 1H), 3.54-3.46 (m, 2H), 3.33 (ddd, *J* = 4.4, 11.7 Hz, 1H), 2.40 (dt, *J* = 12.8, 4.5 Hz, 1H), 2.28 (dt, *J* = 4.5, 13.3 Hz, 1H), 2.10 (s, 3H), 1.56-1.33 (m, 2H); in agreement with literature. HPLC (OD-H, 1.0 mL/min, iPrOH/hexane, 1/9, 20 µL): retention times (min): 10.017 and 11.025.



(-)or(+)(1*R*,3*S*,4*S*,6*R*)-1,3-Diazido-4-*O*-acetyl-cyclohexan-6-ol (31)

Compound **30** (6 mg, 0.02 mmol) was dissolved in toluene (1 mL) and a phosphate buffer (pH 6.7, 1 mL) and *Candida Cylindracea* Lipase (CCL, 6.8 mg) were added. Shake for 72 hours at 30 °C. Filtrate over Celite (flush with excess EtOAc) and evaporate the solvent to yield **31** (5.1 mg, 99%).[1] HPLC (OD-H, 1.0 mL/min, *i*-PrOH/hexane, 1/9, 20 µL): retention times (min): 10.904 (ee > 99%).



(-)or(+)(1*R*,3*S*,4*S*,6*R*)-1,3-Diazido-4-*O*-acetyl-cyclohexan-6-ol (31)

Compound **30** (10.2 mg, 0.036 mmol) was dissolved in toluene (1 mL) and add phosphate buffer (pH 6.2, 1 mL) and *Candida Antarctica* Lipase (CCL, 11.7 mg). Shake for 120 hours

at 30 °C. Filtrate over Celite (flush with excess EtOAc) and evaporate the solvent to yield **31** (8.3 mg, 94%). (OD-H, 1.0 mL/min, *i*PrOH/hexane, 1/9, 20 μL): retention times (min): 11.106 (ee > 99%). $[\alpha]_D^{20}$ -22.9 (c 0.75; CH_2Cl_2).

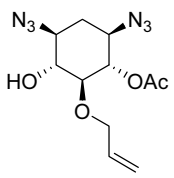
Table 5. Desymmetrization of 1,3-DACH *via* enzymatic resolution with commercially available lipases

substrate	enzyme[b]	enzyme (mg)	method[a]	t (h)	product	yield (%)	ee (%)
30	CAL (fluka)	11.7	A	120	**31**	95	99
19	CAL (fluka)	10.5	A	148	-		
30	CCL	6.8	A^2	72	**31**	99	99
30	PPL	6.5	A^2	72	-		
15	CAL	6.8	A^2	144	-		
15	CCL	6.2	A^2	144	-		
15	PPL	5.8	A^2	144	-		
15	PCL	6.3	A^2	312	-		
15	PFL	5.9	A^2	312	-		
15	RAL	7.1	A^2	144	-		
19	MML	13.7	A^2	240	-		
19	PCL	6.7	A^2	240	-		
19	PFL	12.2	A^2	240	-		
19	RAL	6.7	A^2	240	-		
19	RNL	6.5	A^2	240	-		
19	PPL	14.7	A^2	240	-		
19	AspL	11.2	A^2	240	-		
19	CCL	6.3	A^2	240	-		
30	AspL	6.3	A^2	168	-		
30	MML	5.8	A^2	168	-		
30	PCL	5.7	A^2	168	-		
30	PFL	6.3	A^2	168	-		
30	RAL	6.4	A^2	168	-		
30	RNL	6.2	A^2	168	-		
15	Novo 435	5.3	A^2	480	-		
19	Novo 435	5.5	A^2	480	-		
30	Novo 525	10	A^2	480	-		
15	Novo 525	10	A^2	480	-		
19	Novo 525	10	A^2	480	-		
30	PPL	26.6	A^3	264	-		
15	PPL	25.4	A^3	264	-		
19	PPL	25.8	A^3	264	-		

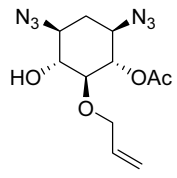
16	CAL	4.8	A[4]	120	-		
16	CCL	5.4	A[4]	120	-		
17	PPL	6.3	B	72	-		
17	CCL	4.9	B	72	**31**	8%	nd
17	CAL	5.7	B	72	**31**	34%	nd
17	Novo 435	5.5	B	72	-		
17	Novo 525	10	B	72	-		
14	PPL	4.8	B	72	-		
14	CCL	6.4	B	72	-		
14	CAL	4.8	B	72	-		
14	Novo 435	4.8	B	72	-		
14	Novo 525	10	B	72	-		
1	PPL	5	B	72	-		
1	CCL	6.9	B	72	-		
1	CAL	4.5	B	72	-		
1	Novo 435	6.8	B	72	-		
1	Novo 525	10	B	72	-		

[a] A: solv. = phosphate buffer pH 6.2/toluene, 1/1; A[2]: solv. = phosphate buffer pH 6.7/toluene, 1/1; A[3]: solv. = phosphate buffer pH 7.0/toluene, 1/1; A[4]: solv. = phosphate buffer pH 6.2/toluene, 1/1; B: solv. = toluene, reactant = AllOAc

[b] CAL = *Candida Antarctica* Lipase; PCL = *Pseudomonas Cepacia* Lipase; PPL = *Porcine Pancreas* Lipase; CCL = *Candida Cylindracea* Lipase; AspL = *Aspergillus* Lipase; RAL = *Rhizopus Arrhizus* Lipase; MML = *Mucor Miehei* Lipase; PFL = *Pseudomonas Fluorescens* Lipase; RNL = *Rhizopus Niveus* Lipase; Novo 435 = Novozym 435 (immobilized CalB); Novo 525 = Novozym 525 (CalB solution).



(±)-(1*R*,2*S*,3*S*,4*R*,6*S)*-4,6-Diazido-2-allyloxycyclohexane-3-acetyloxy-1-ol (34)
Compound **16** (32 mg, 0.094 mmol) was dissolved in THF (1 mL) and cooled to 0 °C. LiOH (4 mg, 0.19 mmol) was dissolved in water (100 µL) and added slowly to the substrate. Stir for 8h hours at 0 °C. Evaporate the solvent and purify by flash column chromatography (EtOAc/*n*-heptane, 1/15 to 1/5) to yield **34** (8 mg, 28%, 91% BORSM). [1] HPLC (AD-H, 1.0 mL/min, *i*-PrOH/hexane, 3/97, 5 µL): retention times (min): 45.190 and 48.168



(+ or -)-(1*R*,2*S*,3*S*,4*R*,6*S)*-4,6-Diazido-2-allyloxycyclohexane-3-acetyloxy-1-ol (34)
Compound **16** (30 mg, 0.088 mmol) was dissolved in MeCN (200µL) and add phosphate buffer (pH 7.5, 2 mL) and Diversa esterase 5 (20 mg).[28] Shake the reaction for 72 hours at 37 °C. Filtrate over Celite (flush with excess EtOAc) and evaporate the solvent to yield **34** (15 mg, 88% BORSM, 58%, 10 mg starting material). R_f 0.40 (EtOAc/*n*-heptane, 1/2). IR υ_{max} film: cm^{-1} 2099, 1743, 1372, 1231, 1070, 1032, 610. [1] H NMR ($CDCl_3$, 300 MHz, ppm): δ 5.9-5.78 (m, 1H) 5.29 (br s, 1H), 5.19-5.14 (m, 1H), 4.94 (t, J = 9.9 Hz, 1H), 4.18 (d, J = 5.4 Hz, 2H), 3.49 (q, J = 9.6, 18.9 Hz, 1H), 3.47-3.3.34 (m, 2H), 3.26 (t, J = 9.3 Hz, 1H), (dt, J = 4.5, 13.5 Hz, 1H), 2.14 (s, 3H), (q, J= 12.3, 25.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 169.8, 134.3, 117.8, 81.3, 76.2, 75.1, 74.2, 59.9, 58.7, 32.1, 21.0. $[\alpha]_D^{20}$ +5.54 (c 0.65; CH_2Cl_2).[1] HRMS (CI) *m/z* calcd for $C_{11}H_{17}O_4SiN_6$ $(M+H)^+$: 297.1311, found:297.1325. HPLC (AD-H, 1.0 mL/min, *i*-PrOH/hexane, 3/97, 5 µL): retention time (min): 47.433 (ee > 99%).

4.11 References

[1] Vourloumis, D.; Winters, G. C.; Simonsen, K. B.; Ayida, B. K.; Shandrick, S.; Zhao, Q.; Hermann, T. *ChemBioChem* **2003**, *4*, 879-885.

[2]. Takano, S.; Higashi, Y.; Kamikubo, T.; Moriya, M.; Ogasawara, K. *Synthesis*, **1993**, 948-950.

[3] Takano, S; Moriya, M.; Ogasawara, K. *Synlett* **1993**, 601-602.

[4] It has to be noted that, although not useful for our purpose, conversion of **3** into (-)-enone **6** is also feasible in only two steps by executing the palladium-catalyzed migration immediately after acetylation.

[5] Trost, B.M.; Surivet, J.P. *Angew. Chem. Int. Ed.*, **2000**, *39*, 3122-3124.

[6] The absolute configuration is assigned by analogy.[5] Further support comes from comparison of the HPLC chromatograms of the products (**6**) obtained by the two different methodologies.

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[8] Recipe for the preparation of 1 kg of AD-mix-α or AD-mix-β: potassium osmate ($K_2OsO_2(OH)$, (0.52 g) and (DHQ),-PHAL (for AD mix-α) *(5.52* g) or $(DHQD)_2$-PHAL (for AD-mix-β) were ground together to give a fine powder, then added to powdered $K_3Fe(CN)$, (700.0 g) and powdered K_2CO_3 (294.0 g), and finally mixed in a blender in a dry box for about 30 min. The resulting mixture should be kept dry and is ready for use. These two AD-mixes are now available from Aldrich. (The 1.4 g of AD-mix-β, necessary for conversion of 1 mmol of the olefin, contains 0.980 g of $K_3Fe(CN)_6$ (3.0 mmol), 0.410 g of K_2C0_3 (3.0 mmol), 0.0078 g of $(DHQD)_2$-PHAL (0.01 mmol), and 0.00074 g of K_2-$OsO_2(OH)$, (0.002 mmol).

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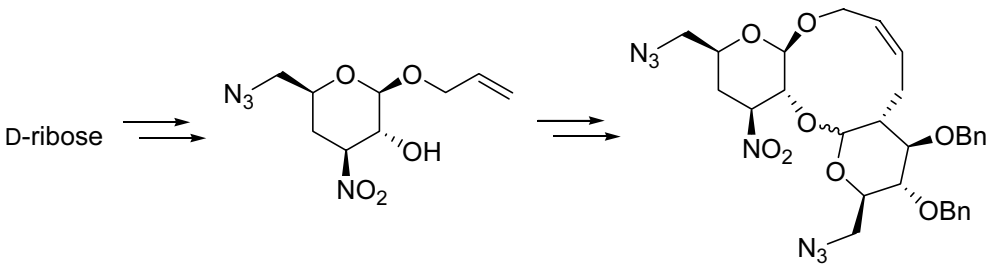
5 A carbohydrate mimic of 2-deoxystreptamine for the preparation of conformationally constrained aminoglycosides

Abstract

The synthesis of a carbohydrate mimic of 2-deoxystreptamine (2-DOS) is described here. Starting from D-ribose, crucial steps of the synthesis involve a nitro aldol condensation and deoxygenation via elimination of an acetate group followed by *in situ* reduction. Moreover, glycosylation of the carbohydrate 2-DOS derivative with phenyl thioglycoside in the presence of TTBP and AgOTf followed by ring closing metathesis yielded a conformationally restricted protected aminoglycoside analogue.



5.1 Introduction

As a consequence of the fact that it is the central scaffold of nearly all aminoglycoside antibitiocs, the diaminocyclohexitol called 2-deoxystreptamine (2-DOS) is a logical starting structure for the development of novel aminoglycosides. For such an endeavor, however, substantial amounts of 2-DOS must be readily available and although many synthetic routes toward 2-DOS have been developed, as for example in the preceding chapters, the disadvantage of most of these routes is the large number of synthetic steps required to synthesize the diaminocyclohexitol ring. From this observation, it was reasoned that a carbohydrate-derived entity like compound **A** (Figure 1) could be an interesting alternative for 2-DOS, taken into consideration that the amino group at *C*-1 is modified in several clinically used aminoglycosides and the *C*-6 hydroxyl is not essential for antibacterial activity (2-DOS numbering). Such a synthesis should be readily achievable from a cheap carbohydrate precursor and, consequently, lead to enantiomerically pure product. To date, only two syntheses of such carbohydrates analogues are known. A diaminocarbohydrate was synthesized starting from benzyl-α-D-glucopyranoside by Meyer zu Reckendorf and coworkers (not depicted),[1] in a ten-step synthesis leading to a diaminocarbohydrate quite similar to 2-DOS, but as a mixture of anomers.[2] The observation that carbohydrates could be a good alternative for 2-DOS was also brought about by Boons co-workers (see also §5.3).[3]

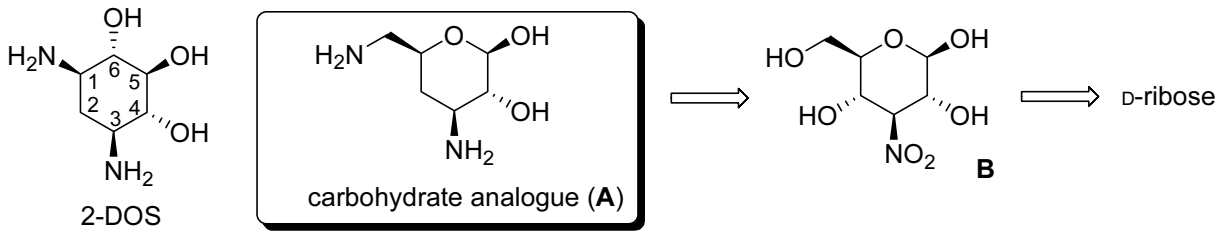


Figure 1. Retrosynthesis of carbohydrate derivative (**A**) as a mimic for 2-deoxystreptamine.

Retrosynthetic analysis of β-configured carbohydrate analogue **A** suggests a synthesis from nitroglucopyranoside **B**, by means of deoxygenation at *C*-4, reduction of the 3-nitro, and azide introduction *C*-6 (carbohydrate numbering). The nitroglucopyranoside in turn was projected to result from a dialdehyde derived from D-ribose,[4] a cheap and enantiopure carbohydrate, via double Henry reaction with nitromethane. Although it is clear that the final carbohydrate analogue lacks the *C*-6 hydroxyl, the fact that the 6-OH of 4,5-linked aminoglycoside antibiotics is only involved in indirect binding events with the RNA backbone (Figure 2)[5,6] led us to assume it could be omitted without severe penalty in binding enthalpy. Furthermore, the amino group on the carbohydrate is

one carbon further away from the cyclitol, but it was surmised that extension of the hydroxyl would not *a priori* be unfavorable, given some precedented aminoglycoside analogues.

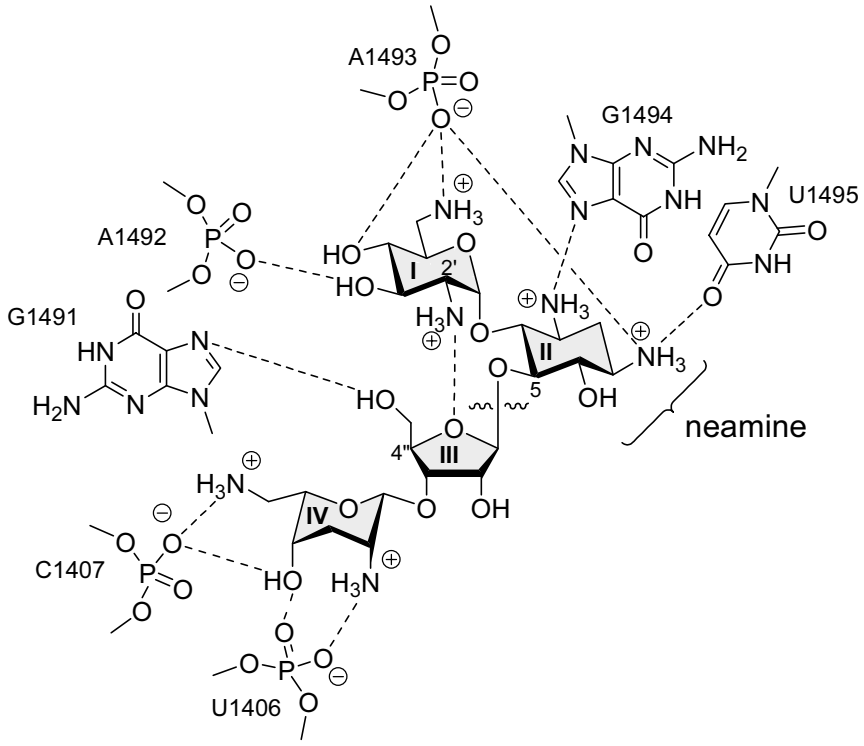


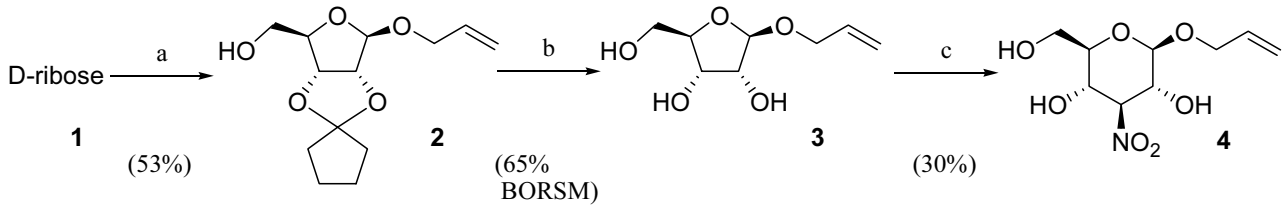
Figure 2. Neomycin complexed to nucleobases and the phosphate backbone of 16S rRNA.[5,6]

5.2 Preparation of 2-DOS carbohydrate mimic

The first synthetic step in the preparation of carbohydrate analogue **A** is triple protection of D-ribose. In a one-pot reaction, both *C*-2 and *C*-3 hydroxyls were protected with a cyclopentylidene group and the 1-hydroxyl with an allyl group yielding the protected β-allyl ribofuranoside **2**. The choice for the cyclopentylidene lies in the fact that various acidic conditions failed to cleanly remove the commonly employed isopropylidene group at this position and give acceptable amounts of product,[7,8,9,10] which led us to a paper by Van Heeswijk and co-workers on the stability of a variety of –ylidene groups.[11,12] Indeed, removal of the cyclopentylidene with CH_3COOH/H_2O (80/20) led to β-*O*-allyl ribofuranoside (**3**) in acceptable yield although also in this case it turned out mandatory to quench the reaction after 48 hours to avoid hydrolysis of the anomeric protective group. Investigations on the synthesis of the β-*O*-allyl ribofuranoside **3** in a single synthetic step by Fisher glycosidation of D-ribose with allyl alcohol activated by $CuSO_4$ and H_2SO_4, $SnCl_4$ or Amberlite IR-120 (H^+, ion-exchange resin) did not improve matters since the α-ribofuranoside was always obtained as the main product.

Crucial step in the synthetic sequence was oxidative cleavage of the diol to a dialdehyde, followed by nitroaldol condensation with nitromethane. Because three new asymmetric centers are formed in this transformation, eight stereoisomeric nitro glycosides could theoretically be formed, apart from additional stereoisomers engendered by epimerization of α-carbons. The fact that the desired stereoisomer **4** has the all-equatorial configuration was an important stimulus to undertake this endeavor and we were delighted to find that a single product 3-nitro-β-D-allylpyranoside (**4**) was isolated after acidification and crystallization from EtOAc (Scheme 1).[13] Two other isomers, formed in smaller proportions, were not isolated. It was found that performing the reaction in an aqueous solution of sodium hydroxide led to lower yields and predominant formation of another isomer.

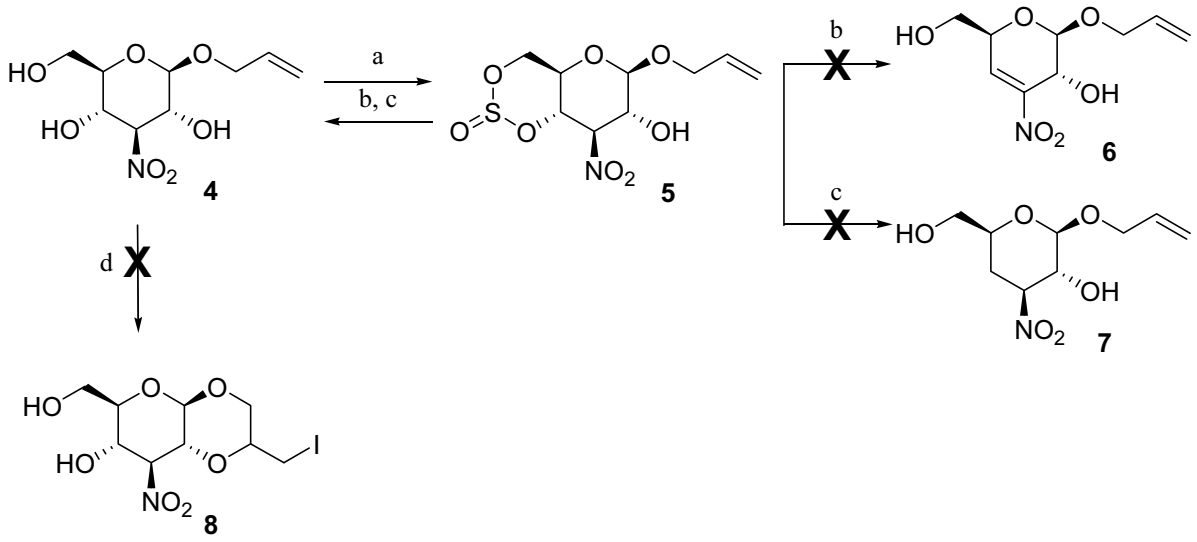
Scheme 1.



Reagents and conditions: (a) $CuSO_4$, cyclopentanone, allyl alcohol, H_2SO_4, 40 °C, 48h; (b) CH_3COOH 80%, 40 °C, 48h; (c) $NaIO_4$, H_2O, 0 °C to rt, 30 min then NaOMe, NO_2Me, MeOH, rt, 45 min.

Having the requisite nitrosugar **4** at hand, regioselective deoxygenation was investigated. Initially, it was expected that such a transformation could be induced via conversion of the 4,6-diol into a cyclic sulfite (**5**) followed by base-effected elimination reaction. Indeed, reaction of **4** with thionyl chloride smoothly led to the desired cyclic sulfite **5** but only starting material **4** was isolated upon subsequent attempted elimination with DBU (Scheme 2). Other bases were also investigated such as $NaHCO_3$ and an elimination reduction procedure with $NaBH_4$, however under all these reaction conditions only nitroglucopyranoside **4** was isolated. A different strategy was followed next, involving a regioselective protection of the 2-hydroxyl by iodoetherification with the *O*-allyl group.[14] Such a methodology was developed by Cipolla and co-workers for allyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside to afford a cyclic iodoether upon treatment with I_2. Unfortunately, upon reaction of the glucopyranoside with I_2 no conversion of starting material was detected even when Et_3N was added or when the reaction temperature was increased.

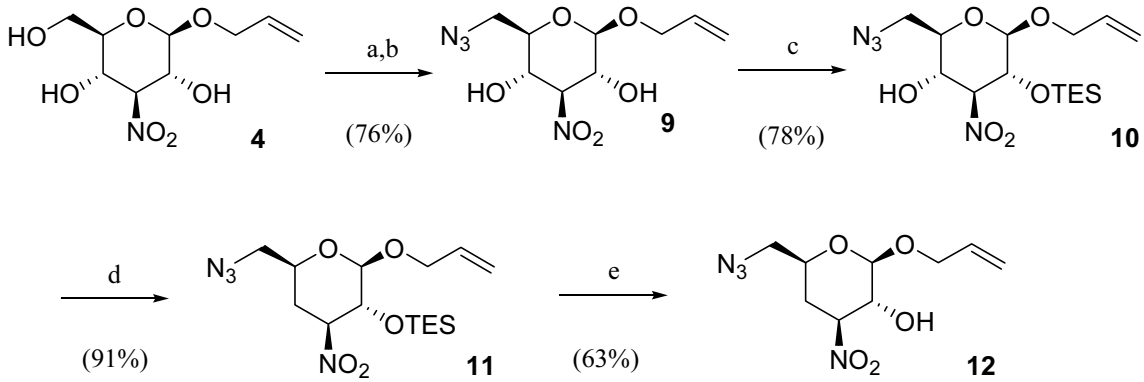
Scheme 2.



Reagents and conditions: (a) $SOCl_2$, pyridine, EtOAc, 0 °C, 2h; (b) DBU, CH_2Cl_2 and $NaHCO_3$; (c) $NaBH_4$, EtOH, 0 °C, o.n.; (d) I_2, Et_3N, THF, rt to heating 50 °C.

Given the unsuccessful attempts described above, our next approach involved prior conversion of the 6-OH into an azide followed by regioselective discrimination of the resulting diol. Thus, treatment of *O*-6 with tosylchloride followed by displacement with sodium azide gave the 6-azidopyranoside **9** in good yield (Scheme 3). Much to our satisfaction, subsequent protection of the 2-hydroxyl with a triethylsilyl group proceeded uneventfully to yield monosilylated glucopyranoside **10**.[15] Subsequent acetylation of *O*-4 with acetylchloride[16] and addition of sodium borohydride to the crude reaction product lead to a one-step elimination-reduction reaction.[17] Finally, removal of the TES group with a 2N HCl solution in EtOAc afforded the carbohydrate mimic of 2-DOS **12** in a total of 8 synthetic steps and an overall yield of 6.6%.

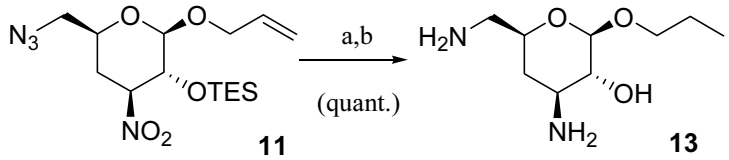
Scheme 3.



Reagents and conditions: (a) TsCl, CH_2Cl_2/ pyridine (1/1), 0 °C to rt, o.n.; (b) NaN_3, DMF, 55 °C, o.n.; (c) TESOTf, Et_3N, CH_2Cl_2, 0 °C, 30 min; (d) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 30 min, then $NaBH_4$, EtOH, rt, 2h; (e) 2N HCl, EtOAc, rt, 10 min.

Before further application of 12 was undertaken, reduction of the nitro or azido group was investigated. Indeed, hydrogenation of nitroglucopyranoside 12 was achieved upon reaction with Ra-Ni, leading to reduction of azido, nitro and the allyl group. Subsequent removal of the TES group under aforementioned conditions yielded the carbohydrate analogue 13 quantitatively.

Scheme 4.



Reagents and conditions: (a) Ra-Ni, MeOH, H_2, 3 bar, rt, 24h; (b) 2N HCl, EtOAc, rt, 10 min.

5.3 Conformationally constrained aminoglycoside ligand

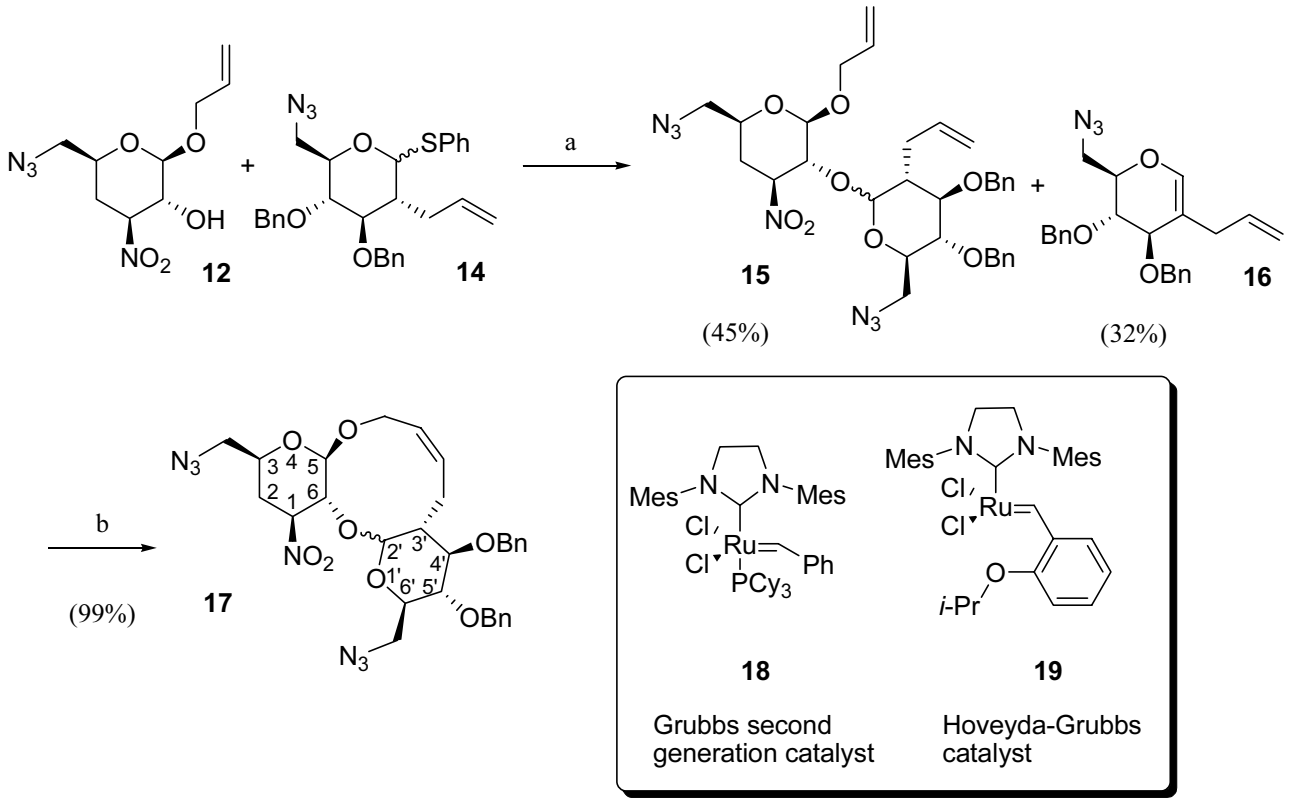
NMR spectroscopy and x-ray crystallography studies have indicated that neamine is the minimal motif for selective binding to the A-site of 16S rRNA.[5,6] It has also been shown that 2,6-diamino-2,6-dideoxyglucopyranoside makes many of the key interactions with RNA. Studies by Wong and co-workers have demonstrated that substitution patterns other than 2,6-diamines lead to loss of activity of these antibiotics.[18] Studies by Boons and co-workers[3] on a library of 24 disaccharides mimics of neamine including α(1-3) or β(1-3) and α(1-4) or β(1-4) linked dimers containing 2-4 amino groups indicated that the compound with best binding affinity was the one with 4 amino groups (*C*-2, *C*-2', *C*-6, *C*-6'), displaying an affinity similar to that of neamine. These results stimulated us to investigate incorporation of nitro analogue **12** in a conformationally restricted analogue of neamine *via* coupling to a thioglycoside followed by macrocycle formation. Extensive clinical use of the aminoglycosides is limited, due to the global development of microbial resistance as the result of structural modification by bacterial enzymes.[19,20] Another drawback of the aminoglycosides is the associated nephro- and ototoxicities.[21] It is also known that most aminoglycosides can bind to a variety of RNA targets with lack of high selectivity. The NMR structure derived from paromomycin complexed to 16S rRNA revealed that several intermolecular contacts between the aminoglycosides and RNA recognition sides are important for binding.[5a] Our attention was drawn to a hydrogen bond between *N*-2' and *O*-4" (Figure 2) and it was reasoned that a neamine analogue covalently linked between *C*-3' and *O*-5 would be locked in the appropriate conformation for binding to rRNA and may therefore show enhanced binding specificity (Scheme 5, 2-DOS numbering). Along the same line of reasoning, Jiménez-Barbero and co-workers recently prepared a conformationally restricted neomycin B analogue and found it to be less susceptible to

resistance.[22] Another recent report on the synthesis of conformationally restricted analogues of both neomycin and paromomycin was also published this year by Blount *et al.*[23]

Thus, glycosylation of the carbohydrate acceptor **12** with donor **14**[24] was carried out in the presence of 2,4,6-tri-tert-butylpyrimidine (TTBP) and AgOTf (Scheme 5).[25] The product was formed in a yield of 45% and the α/β ratio was around 3:1.[26,1] Apart from that, (unavoidable) formation of a side product was observed, NMR analysis of which revealed that elimination product (**16**) was formed in a yield of 32%. Next, after separation of the two products (**15** and **16**) ring-closing metathesis reaction was performed. Unfortunately, exposure of the dicarbohydrate (**15**) to the second generation of Grubbs' catalyst to induce ring-closing metathesis did not yield the desired product, presumably caused by incompatibility of the azides with the ruthenium phosphine ligand.[27] The undesired Staudinger reduction could be simply avoided by prior reduction of the azido groups followed by protection of the corresponding amine groups. However, a more elegant approach avoiding the requisite additional two steps, involved the use of the phosphine-free Hoveyda–Grubbs catalyst **19**. Much to our satisfaction reaction of compound **15** with the Hoveyda-Grubbs catalyst indeed afforded the cyclic product **17** in a high yield of 99%.

Although no further chemistry was performed with the resulting product **17** at this stage, it is assumed that reduction and deprotection of derivative **17** can be performed under the aforementioned conditions (Ra-Ni, H2).

Scheme 5.



Reagents and conditions: (a) AgOTf, 4Å MS, TTBP, CH_2Cl_2, 0 °C to rt, o.n. (α /β ≈ 1/3); (b) Hoveyda's catalyst, toluene, rt, 4h.

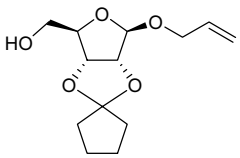
5.4 Concluding remarks

A carbohydrate analogue of 2-DOS was synthesized successfully in 8 steps and an overall yield of 6.6%. The analogue is enantiopure and orthogonally protected. Model reduction and deprotection of the analogue proceeded easily in only two steps in a high yield. More importantly, the carbohydrate precursor is conveniently protected for incorporation in new aminoglycoside entities, suited for the synthesis of 4,5-linked aminoglycoside antibiotics. Moreover, the allyl at *O*-1 makes the carbohydrate mimic highly suitable for the synthesis of conformationally restricted RNA binders. In addition, the carbohydrate derivative was coupled successfully with thioglycoside **14**, to form neamine mimic **15**. A conformationally restricted analogue was synthesized via a ring-closing metathesis with the Hoveyda-Grubbs catalysts. Deprotection has to be performed before biological tests can be achieved.

5.5 Acknowledgements

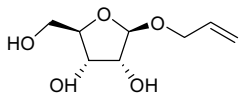
B. A. M. W. van den Broek was gratefully acknowledged for the glycosylation of the 2-DOS carbohydrate precursor **12** with thioglycoside **14**.

5.6 Experimental Section



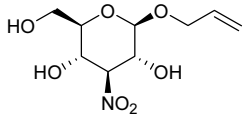
Allyl-2,3-*O*-cyclopentanone-β-D-ribofuranoside (2)

Powdered D-ribose (**1**, 5.85 g, 38.9 mmol) and anhydrous cuprous sulfate (12.4 g) were suspended in a mixture of cyclopentanone (110 mL) and allyl alcohol (32 mL) containing a catalytic amount of H_2SO_4 (0.2 mL). The resulting mixture was stirred at 40 °C for 48 h and then neutralized with $NaHCO_3$, filtered and the solvents were evaporated. The crude product was extracted with EtOAc and washed with brine, dried (Na_2SO_4), and the solvent was evaporated to give the crude product which was purified by flash chromatography (EtOAc/*n*-heptane, 1/10), to obtain **2** (3.32 g, 53%) as a colorless oil. [12] $[\alpha]_D^{20}$ -70.8 (c 0.35; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 3475, 2964, 1338, 1102, 1043, 1001, 914, 742. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.90 (m, 1H, Allyl), 5.30 (m, 2H, Allyl), 5.13 (s, 1H, CH), 4.78 (d, 1H, *J* = 6.0 Hz, CH), 4.55 (d, 1H, *J* = 6.0 Hz, CH), 4.41 (br s, 1H, OH), 4.21 (m, 1H, CH), 4.08 (m, 1H, CH), 3.69 (m, 2H, CH), 3.22 (m, 1H, CH), 1.68 (m, 8H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 132.9, 121.6, 118.2, 107.6, 88.1, 85.6, 81.3, 68.8, 63.9, 35.6, 35.5, 23.5, 23.0. HRMS (EI) *m/z* calcd for $C_{13}H_{20}O_5$ $(M)^+$: 256.1311, found: 256.1312.



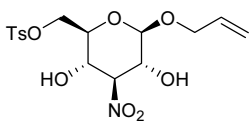
Allyl-β-D-ribofuranoside (3)

Compound **2** (2.05 g, 8.00 mmol) was dissolved in 80 % acetic acid and heated to 40 °C for 48 h. The reaction mixture was neutralized with $NaHCO_3$ and evaporated flash column chromatography (EtOAc/*n*-heptane, 1/5) gave **3** as colorless oil (604 mg, 40%, BORSM 65%). R_f 0.38 (EtOAc). $[\alpha]_D^{20}$ -57.1 (c 0.77; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 3347, 2926, 1640, 1084, 1033, 993, 931, 633, 559. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.04-5.97 (m, 1H, allyl), 5.28 (dd, *J* = 1.6, 1.8 Hz, 1H, allyl), 5.20 (dd, *J* = 1.8, 1.6 Hz, 1H, allyl), 4.97 (s, 1H, CH), 4.20-3.91 (m, 7H, CH), 3.72-3.59 (m, 2H, OH), 3.67 (br s, 1H, OH). ^{13}C NMR (CD_3OD, 75 MHz, ppm): δ 135.4, 116.8, 107.7, 84.8, 76.2, 72.7, 69.1, 65.0. HRMS (CI) *m/z* calcd for $C_8H_{15}O_5$ $(M+H)^+$: 191.0919, found: 191.0920.



Allyl-3-nitro-3-deoxy-β-D-glucopyranoside (4)

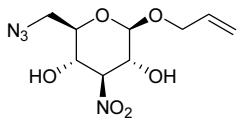
$NaIO_4$ (7.68 g, 35.9 mmol) was dissolved in H_2O (90 mL). After cooling the reaction mixture to 0 °C, compound **3** (6.83 g, 35.9 mmol) was added and the reaction mixture was carefully heated to rt. Note the $NaIO_4$ is only dissolved at rt. The oxidation is finished after stirring for 30 min according to TLC analysis in EtOAc. EtOH was added to the reaction mixture and cooled to 0 °C and filtered to get rid of the $NaIO_4$. The filtrate was evaporated and dissolved in EtOH and filtered again. The reaction mixture was now evaporated twice with MeOH. The obtained dialdehyde was dissolved in MeOH and after cooling nitromethane (2.0 mL, 37.34 mmol) was added. Freshly prepared NaOMe (2.48 g, 35.9 mmol) was now added and the reaction was stirred at rt for 45 min. To the reaction mixture was added to a cold solution of Amberlite IR-120 (H^+) in MeOH. The yellow reaction mixture becomes colorless after stirring for approximately 10 min. The reaction mixture was passed through a column of Amberlite and thoroughly rinsed with MeOH. After evaporation twice with EtOAc the reaction mixture was stored in the fridge for one night after which the EtOAc was filtered off to yield a white powder of compound **4** (2.54 g, 30%). R_f 0.62 (EtOAc). Mp: 158.6 °C. $[\alpha]_D^{20}$ -24.9 (c 1.0; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 3285, 1556, 1124, 1079, 1042, 1012. ^1H NMR (CD_3OD, 400 MHz, ppm): δ 7.09-5.96 (m, 1H, CH), 5.37 (ddt, *J* = 1.6, 1.8 Hz, 1H, CH), 5.31 (ddt, *J* = 1.3, 1.5 Hz, 1H, CH), 4.46 (t, *J* = 10.1 Hz ,1H, CH), 4.48-4.33 (m, 1H, CH_2), 4.36 (d, *J* = 2.5 Hz, 1H, CH), 4.21-4.13 (m, 1H, CH_2), 3.92 (t, *J* = 9.8 Hz, 1H, CH), 3.90-3.51 (m, 3H, CH), 3.38-3.16 (m, 1H, CH). ^{13}C NMR (CD_3Cl_3, 75 MHz, ppm): δ 135.1 (CH), 117.3 (CH_2), 102.7 (CH), 95.8 (CH), 78.1 (CH), 72.3 (CH), 71.1 (CH_2), 69.2 (CH), 62.0 (CH_2). HRMS (ESI) *m/z* calcd for $C_9H_{15}O_7N$ $(M+Na)^+$: 272.0746, found: 272.0745. Elemental analysis: calculated for ($C_9H_{15}O_7$) C 43.37, H 6.07, N 5.62, found C 43.00, H 6.10, N 5.51.



Allyl-3-nitro-3-deoxy-6-*O*-tosyl-β-D-glucopyranoside

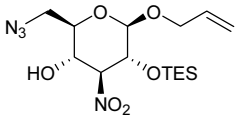
Compound **4** (1.07 g, 5.13 mmol) was dissolved in a mixture of (CH_2Cl_2/pyridine, 1/1, 0.2 mL). After cooling the mixture to 0 °C tosylchloride (1.17 g, 6.16 mmol) was added. The reaction was stirred overnight. The reaction mixture was quenched with water and extracted with EtOAc, dried with Na_2SO_4. To yield after flash column chromatography (EtOAc/*n*-heptane, 1/10) the compound as a colorless oil (1.74 g, 91%). R_f 0.78

(EtOAc). IR υ_{max} film: (cm^{-1}) 3482, 1559, 1173, 1039, 981, 814, 730, 533. ^1H NMR (CD_3OD, 400 MHz, ppm): δ 7.79, (d, *J* = 8.2 Hz, 2H, arom), 7.35 (d, *J* = 8.1 Hz, 2H, arom), 5.94-5.82 (m, 1H), 5.33-5.19 (m, 2H), 4.54 (t, *J* = 10.1 Hz, 1H), 4.41-4.33 (m, 2H), 4.34-4.25 (m, 2H), 4.18-4.03 (m, 3H), 3.94 (dq, *J* = 9.7, 4.1, 2.1 Hz, 1H), 3.57-3.51 (m, 1H), 1.54 (s, 3H). ^{13}C NMR (CD_3Cl_3, 75 MHz, ppm): δ 145.3, 132.9, 130.0, 128.0, 118.6, 101.2, 92.2, 74.1, 70.9, 67.7, 22.1.



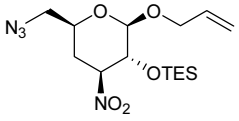
Allyl-6-azido-3-nitro-3,6-dideoxy-β-D-glucopyranoside (9)

Tosyl glucopyranoside (33 mg, 0.082 mmol) was dissolved in DMF (1 mL). The reaction mixture was heated to 55 °C and NaN_3 (10 mg, 0.16 mmol) was added. After stirring overnight the reaction mixture was quenched with water and extracted with EtOAc, to yield after flash column chromatography in (EtOAc/*n*-heptane, 2/3) compound **9** as a white solid (17 mg, 84%). R_f 0.28 (EtOAc/*n*-heptane, 2/3). $[\alpha]_D^{20}$ -16 (c 0.45; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 3426, 2103, 1559, 1372, 1281, 1065. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.04-5.81 (m, 1H), 5.52-5.20 (m, 2H), 4.54 (t, *J* = 10.0 Hz, 1H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.36 (d, *J* = 7.5 Hz, 1H), 4.21-4.30 (m, 2H), 4.18 (t, *J* = 9.5 Hz, 1H), 3.63 (br 1, 1H, OH), 3.59-3.38 (m, 3H), 3.24 (br s, 1H, OH). ^{13}C NMR (CD_3Cl_3, 75 MHz, ppm): δ 132.9, 118.9, 100.9, 92.8, 75.5, 70.9, 70.8, 69.0, 51.2. HRMS (CI) *m/z* calcd for $C_9H_{15}O_6N_4$ $(M+H)^+$: 275.0992, found: 275.0981.



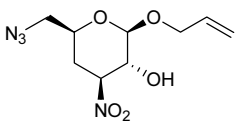
Allyl-6-azido-3-nitro-3,6-dideoxy-2-*O*-triethylsilyl-β-D-glucopyranoside (10)

The glucopyranoside **9** (13 mg, 0.047 mmol) was dissolved in CH_2Cl_2. After cooling the reaction to 0 °C triethylsilyl trifluoromethanesulfonate (16 µL, 0.071 mmol) and Et_3N (17 µL, 1.2 mmol) were added and the reaction mixture was stirred for half an hour and quenched with sat. aq. NH_4Cl and extracted with CH_2Cl_2 and dried with Na_2SO_4. Flash column chromatography (EtOAc/*n*-heptane, 2/3) gave compound **10** (18 mg, 78%) as a colorless oil. R_f 0.52 (EtOAc/*n*-heptane, 1/10). $[\alpha]_D^{20}$ -14 (c 0.46; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 3425, 2876, 2099, 1557, 1067, 815, 745. ^1H NMR ($CDCl_3$, 400 MHz, ppm): 6.05-5.90 (m, 1H), 5.42-5.18 (m, 2H), 4.49 (t, *J* = 6.4 Hz, 1H), 4.45-4.31 (m, 1H, CH), 4.37 (d, *J* = 7.6 Hz, 1H), 4.19-3.92 (m, 3H), 3.52-3.49 (m, 3H), 1.14-0.79 (m, 9H, TES), 072-0.45 (m, 6H, TES).^{13}C NMR (CD_3Cl_3, 75 MHz, ppm): δ 133.1, 118.5, 101.6, 95.0, 75.3, 72.3, 70.9, 69.4, 51.4, 6.9, 5.1. HRMS (FAB) *m/z* calcd for $C_{15}H_{29}O_6N_4Si$ $(M+H)^+$: 389.1856, found: 389.1863.



Allyl-6-azido-3-nitro-3,4,6-trideoxy-2-*O*-triethylsilyl-β-D-glucopyranoside (11)

Compound **10** (100 mg, 0.26 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C. Et_3N (64 µL, 0.47 mmol), Ac_2O (49 µL, 0.51 mmol) and DMAP (cat.) were added and the reaction was stirred for 30 min. The reaction mixture was quenched with a 0.1 M HCl solution and extracted with CH_2Cl_2 and dried with Na_2SO_4. The compound was dissolved in EtOH (2mL) and $NaBH_4$ (20 mg, 0.51 mmol) was added to the reaction mixture. The reaction mixture was stirred at room temperature for 2h and quenched with acetone and evaporated. Flash column chromatography (EtOAc/*n*-heptane, 1/5) gave the compound **11** (87 mg, 91%) as colorless oil. R_f 0.60 (EtOAc/*n*-heptane, 2/3). $[\alpha]_D^{20}$ -29 (c 0.32; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 2954, 2877, 2099, 1558, 1130, 1006, 743. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.10-5.82 (m, 1H, allyl), 5.49-5.18 (m, 2H, allyl), 4.64-4.54 (m, 1H, H_3), 4.46-4.38 (m, 1H, H_{7a}), 4.32 (d, 1H, *J* = 7.6 Hz, H_1), 4.15-4.00 (m, 2H, H7b and H_2), 3.70-3.65 (m, 1H, H_5), 3.62-3.39 (m, 1H, H_{6a}), 2.20 (dd, 1H, *J* = 3.5 Hz, H_{6b}), 2.20 (ddd, 1H, *J* = 1.9 Hz, H_{4a}), 2.04 (q, 1H, *J* = 5.9 Hz, H_{4b}), 1.01-0.81 (m, 9H, TES), 0.71-0.51 (m, 6H, TES). ^{13}C NMR (CD_3Cl_3, 75 MHz, ppm): δ 133.2, 118.4, 101.8, 88.1, 72.5, 71.7, 70.7, 54.0, 33.6, 6.9, 5.2. HRMS (FAB) *m/z* calcd for $C_{15}H_{29}O_5N_4Si$ $(M+H)^+$: 373.1907, found: 373.1904.

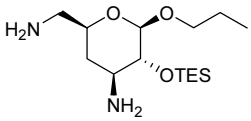


Allyl-3-nitro-3,4,6-trideoxy-6-azido-β-D-glucopyranoside (12)

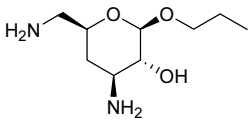
Compound **11** (28 mg, 0.075 mmol) was dissolved in a 2M HCl solution and stirred for 10 min. at room temperature. The reaction mixture was evaporated and flash column chromatography (EtOAc/*n*-heptane, 1/5) gave **12** (12 mg, 63%) as colorless oil. R_f 0.55 (EtOAc/*n*-heptane, 1/1). $[\alpha]_D^{20}$ – 26 (1.4; CH_2Cl_2). IR υ_{max} film: (cm^{-1}) 3431, 2924, 2099, 1556, 1065, 1036. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.07-5.84 (m, 1H), 5.32 (dd, *J* = 35.2, 17.1 Hz, 2H), 4.59 (ddd, *J* = 5.0, 10.0, 12.5 Hz, 1H), 4.38 (d, *J* = 6.6 Hz, 1H), 4.51-4.31 (m, 1H), 4.14 (ddd, *J* = 1.1, 6.5, 12.6 Hz, 1H), 4.01

(dd, *J* = 8.0, 9.7 Hz, 1H), 3.75-3.64 (m, 1H), 3.47 (dd, *J* = 7.2, 13.0 Hz, 1H), 3.21 (dd, *J* = 3.4, 13.0 Hz, 1H), 2.71 (br s, 1H), 2.41-2.19 (m, 1H), 2.02 (q, *J* = 12.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 133.3, 118.8, 101.3, 85.7, 71.8, 71.2, 70.5, 53.8, 33.1. HRMS (CI) *m/z* calcd for $C_9H_{14}O_5N_4$ $(M+H)^+$: 259.1042, found: 259.1049.

Propyl-3,6-diamino-3,4,6-trideoxy-2-*O*-triethylsilyl-β-D-glucopyranoside

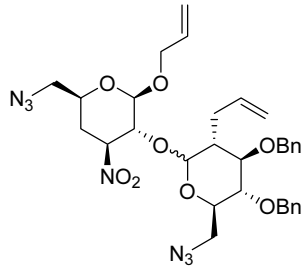


Compound **11** (30 mg, 0.081 mmol) was dissolved in MeOH, Raney Ni in MeOH (7 mL) was added and the hydrogenation was performed on a Parr apparatus overnight at 3 bar, followed by filtration over Hyflo-supercel and evaporation of the solvent to yield compound as a white powder. IR υ_{max} film: (cm^{-1}) 3369, 2955, 1582, 1113, 1078, 823, 741. ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 4.13 (br d, *J* = 7.4 Hz, 1H), 3.77 (dd, *J* = 7.3, 16.4, 1H), 3.57-3.34 (m, 2H), 3.27 (br s, 1H), 3.12-2.94 (m, 1H), 2.79-2.58 (m, 2H), 1.75 (dd, *J* = 3.0, 12.8 Hz, 1H), 1.19 (dq, *J* = 7.2, 14.3 Hz, 2H), 0.92 (dd, *J* = 11.9, 24.2 Hz, 1H), 1.04-0.83 (m, 12H), 0.77-0.58 (m, 6H). ^{13}C NMR (CD_3OD, 75 MHz, ppm): δ 104.6, 79.0, 75.0, 72.0, 54.7, 46.7, 36.5, 24.3, 11.1, 7.4, 6.3. HRMS (ESI) *m/z* calcd for $C_{15}H_{35}O_3N_2Si$ $(M+H)^+$: 319.2417, found: 319.2404.



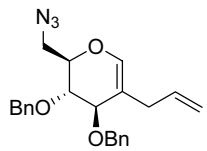
Propyl-3,6-diamino-3,4,6-trideoxy-β-D-glucopyranoside (13)

The diamino glucopyranoside (26 mg, 0.081 mmol) was dissolved in a 2 M HCl solution in EtOAc (5 mL). The reaction mixture was stirred for 1 ½ h and the solvent was evaporated and the residue was dissolved in *t*-BuOH and evaporated this was repeated a two times, to yield compound **13** as a white powder (22 mg, quant, 2 steps). $[\alpha]_D^{20}$ – 3.5 (1.1; H_2O). ^{1}H NMR (D_2O, 300 MHz, ppm): δ 4.53 (d, 1H, *J* = 7.2 Hz), 4.10-3.87 (m, 2H), 3.71-60 (m, 1H), 3.51-3.42 (m, 2H), 3.40-2.30 (m, 1H), 3.22-3.05 (m, 1H), 2.32-2.15 (m, 1H), 1.81-1.58 (m, 3H), 0.95 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (D_2O, 75 MHz, ppm): δ 103.1, 73.3, 71.5, 69.5, 52.3, 43.2, 32.2, 23.2, 10.7. HRMS (CI) *m/z* calcd for $C_9H_{20}O_3N_2$ $(M+H)^+$: 205.1539, found: 205.1552.



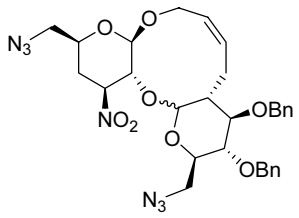
Allyl(2'-*C*-allyl-3',4'-di-*O*-benzyl-2',6',-dideoxy-α/β-D-glucopyranosyl)-(1',2)-6-azido-3,4,6-trideoxy-3-nitro-β-D-glucopyranoside (15)

Flask **1**: AgOTf (142 mg, 0.552 mmol) was co-evaporated three times with toluene and activated molecular sieves (50 mg) were added. Flask **2**: Compound **12** (45 mg, 0.18 mmol) and **14** (93 mg, 0.19 mmol) were co-evaporated three times with toluene after which DCE (3 mL) and TTBP were added (137 mg, 0.552 mmol) and the mixture was stirred for 30 min. Flask **1** with AgOTf was now cooled to 0 °C and flask **2** was added under argon atmosphere. The reaction mixture was stirred o.n. at room temperature. The reaction mixture was quenched with 50 equiv pyridine and filtered through a layer of Hyflo-supercel. The solvents were evaporated and flash column chromatography (EtOAc/*n*-heptane, 1/20 to 1/2) gave product **15** (54 mg, 45%) as colorless oil and **16** (23 mg, 32%) and the starting material (4 mg, 9%) and. R_f 0.45 (EtOAc/*n*-heptane, 1/1). IR υ_{max} film: (cm^{-1}) 2915, 2098, 1557, 1453, 1371, 1283, 1066, 919, 740, 699. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.42-7.21 (m, 10H), 6.09-5.69 (m, 2H), 5.51-5.35 (m, 1H), 5.27-5.21 (m, 1H), 5.15-4.95 (m, 3H), 4.92-4.81 (m, 3H), 4.72-4.65 (m, 4H), 4.49-4.31 (m, 2H), 4.32-4.04 (m, 2H), 3.82-3.62 (m, 1H), 3.51-3.35 (m, 6H), 2.52-2.32 (m, 1H), 2.28-2.10 (m, 2H), 1.91-1.82 (m, 1H). HRMS (FAB) *m/z* calcd for $C_{32}H_{40}O_8N_7$ $(M+H)^+$: 650.2938, found: 650.2918.



1,5-Anhydro-2-allyl-2,6-dideoxy-D-arabino-hex-l-enitol (16)

Compound **16** (23 mg, 32%): R_f 0.75 (EtOAc/*n*-heptane, 1/2). IR υ_{max} film: (cm^{-1}) 3336, 2070, 1120, 1090, 973, 823, 697. ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 7.39-7.23 (m, 10H), 6.23 (s, 1H), 5.81-5.52 (m, 1H), 5.11-4.91 (m, 2H), 4.66 (s, 2H), 4.52 (dd, *J* = 11.5, 45.9 Hz, 2H), 4.22-4.07 (m, 1H), 3.94 (d, *J* = 4.3 Hz, 1H), 3.84 (dd, *J* = 4.3, 5.5 Hz, 1H), 3.65 (dd, *J* = 7.2, 13.2 Hz, 1H), 3.45-3.22 (m, 1H), 2.72 (dq, *J* = 6.3, 15.4 Hz, 2H). ^{13}C NMR (CD_3OD, 75 MHz, ppm): δ 139.4, 135.8, 114.6, 110.5, 75.0, 73.3, 73.3, 71.8, 70.9, 49.4, 32.4. HRMS (EI) *m/z* calcd for $C_{23}H_{26}O_3N_3$ $(M+H)^+$: 392.1974, found: 392.1979.



(2*R*,3*S*,4*R*,4*R*,9*R*,11*S*,13*S*,13*R*)-2,11-bis(azidomethyl)-3,4-bis(benzyloxy)-13-nitro-2,3,4,4,5,8,9,11,12,13,13,14-dodecahydrodipyrano[2,3-2',3'-][1,4]dioxecine (17)

Compound **15** (6 mg, 9 10^{-3} mmol) was co-evaporated three times with toluene and dissolved in toluene (0.5 mL) again. Argon was bubbled through the solution for ten min. To the reaction mixture was added the Hoveyda catalyst (9.8 mg, 15 10^{-3} mmol). The mixture was stirred for 4 h at rt and evaporated. Column chromatography (EtOAc/*n*-heptane, 1/10 to 1/1) gave **17** (6 mg, 99%), as a white solid. R_f 0.34 (EtOAc/*n*-heptane, 1/1). IR υ_{max} film: (cm^{-1}) 2924, 2100, 1557, 1067, 1028, 916. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.39-7.21 (m, 10H), 5.41-4.32 (m, 12H), 4.30-4.04 (m, 2H), 3.81-3.62 (m, 1H), 3.51-3.32 (m, 6H), 2.52-2.32 (m, 1H), 2.28-2.10 (m, 2H), 1.91-1.82 (m, 1H). HRMS (ESI) m/z calcd for $C_{30}H_{35}O_8N_7$ $(M+Na)^+$, 644.2445 found 644.2450.

5.7 References

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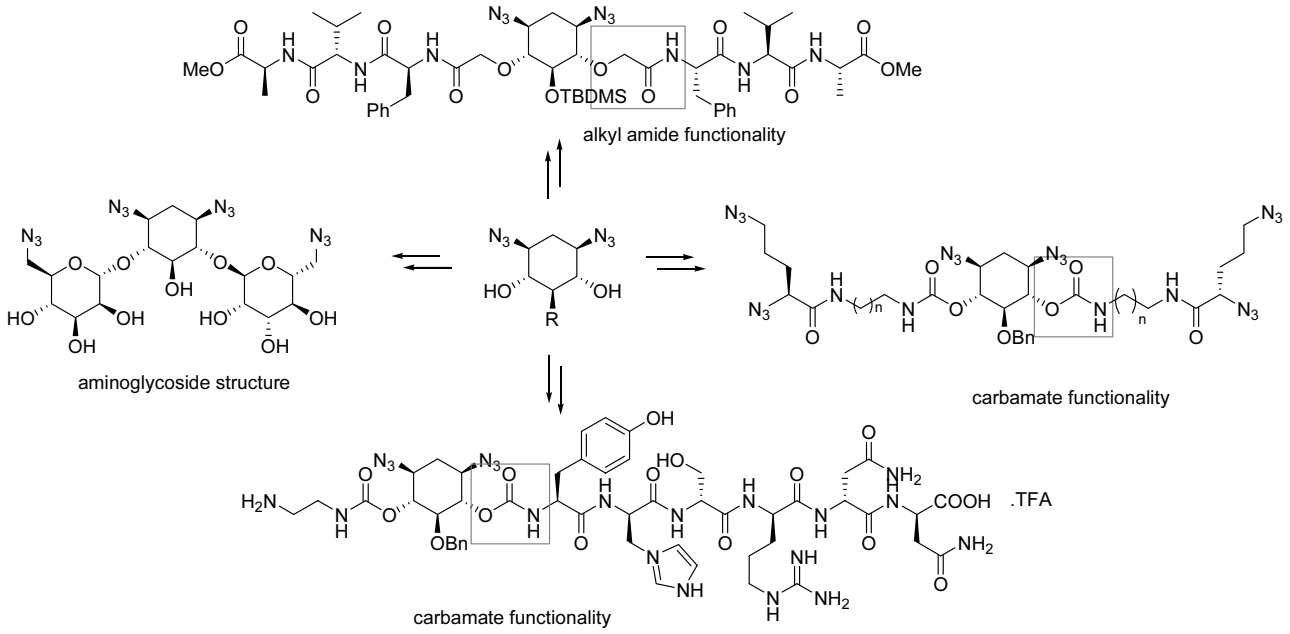
6 4,6-Linked 2-deoxystreptamines as potential RNA binders

Abstract

The synthesis of new aminoglycoside and peptidyl 2-deoxystreptamine (2-DOS) structures is described based on bidirectional functionalization of 2-DOS scaffolds, synthesized in the previous chapters. Aminoglycoside-type structures were synthesized by dual glycosylation of the diazidocyclitol moiety. Peptidyl 2-DOS structures were obtained by coupling amino acids or peptides to the diazidocyclitol scaffold *via* ester, alkyl amide or carbamate functionality.

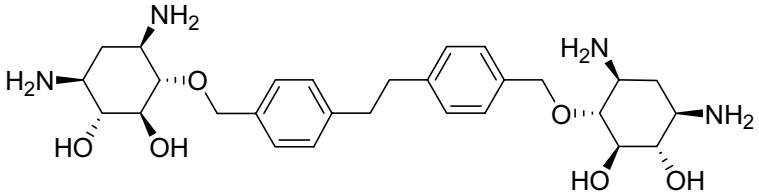
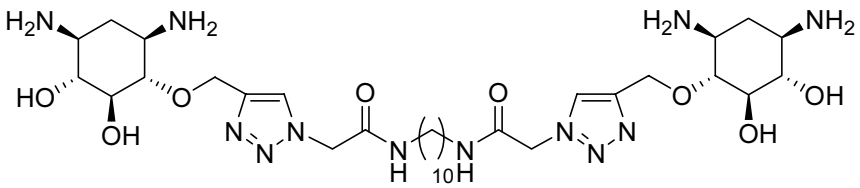


6.1 Introduction

In recent years it has become increasingly apparent that RNA is not a passive bystander but plays an active and essential role in a large number of biological processes. Awareness has grown of the potential to develop tools to target RNA with small organic molecules to find new leads in drug development. Natural products with RNA affinity are the aminoglycosides and therefore are suitable lead compounds for RNA drug development. However, aminoglycosides bind with relatively low affinity and lack sufficient selectivity for a specific RNA sequence. Therefore, efforts to generate various derivatives of aminoglycosides could be a versatile strategy to increase both affinity and selectivity for a specific RNA construct.

The most conserved element of aminoglycosides, the core structure 2-deoxystreptamine (2-DOS), has been reported to bind to two base units within a disrupted RNA helix.[1] Solution studies carried out with 2-DOS have shown that it will be bound toward 5'-3' two-base steps (including GU, UG and GG). Experiments conducted by Hergenrother and co-workers indicated that binding of 2-DOS to specific RNA hairpin loops is low and comparable to kanamycin B (Table 1, entry 1 and 2).[2] Interestingly, they also demonstrated that specific 2-DOS *dimers* bind more tightly than kanamycin in low micromolar range (entry 3). Most recently, it was also shown that several members of a small library of 2-DOS dimers, prepared by Cu(I)-catalyzed variant of the Huisgen 1,3-dipolar cycloaddition of alkynes and azides, were able to bind discriminatively to particular RNA secondary structures with high affinity (entry 4).[3]

Table 1. Binding of 2-DOS dimers to RNA hairpin-loops

entry	heteroconjugate	K_d value (μM)[a]
1	kanamycin A or B, ribostamycin, paromomycin, apramycin	>1000
2	2-DOS	>1000
3		6–16
4		0.07–25

[a] Average binding constant of 2-DOS based constructs to different stem-loops.

The observations above indicate that 2-DOS alone, or at least in oligomeric form, has the capacity to bind to RNA secondary structures. We therefore reasoned that the 2-DOS precursors synthesized in the previous chapters could serve as interesting starting points for the synthesis of new RNA binders. It was expected that conjugation of the aminocyclitol with additional RNA binding pharmacophores could provide novel structures with the requisite flexibility and functionality to bind to RNA (Figure 1). Particularly, trifunctional structures obtained by bidirectional chain-extension may result in high affinity RNA ligands, which can be divided into (I) aminoglycosides and (II) peptidyl 2-DOS structures.

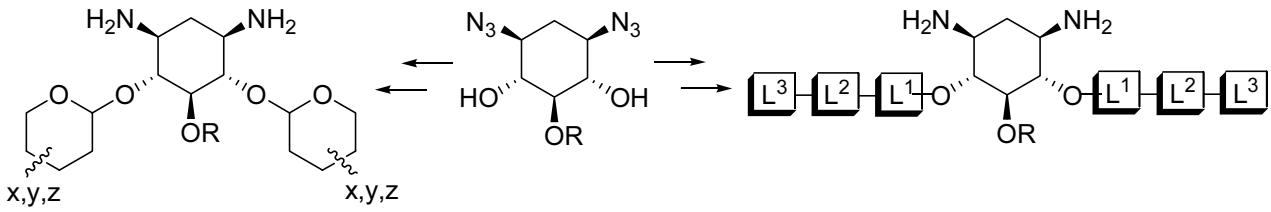


Figure 1. Aminoglycosides and bispeptidyl 2-DOS as potential RNA binders

The following arguments validate the synthesis of such structures:

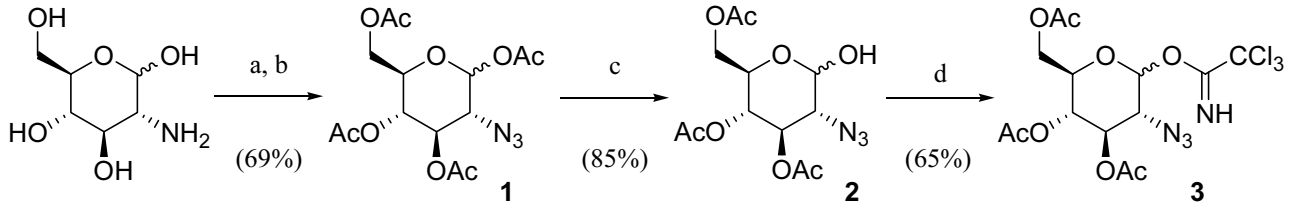
(I) Synthesis of aminoglycoside analogues from the existing antibiotics is cumbersome and laborious due to the plethora of alcohols and amines necessitating extensive protection-deprotection schemes. A straightforward route to non-natural aminoglycoside-like structures involves the simultaneous glycosylation of both alcohols of the 2-DOS azido precursor. This will be described in §6.2.

(II) In order to find highly selective binders for a particular RNA construct, a combinatorial approach is a powerful strategy for variation and diversification of binding elements. The RNA-binding specificity of a molecule is dictated by the same structural elements applicable to protein ligands, such as hydrogen bonding capability, van der Waals and electrostatic interactions, proper stereochemistry, etc. We realized that peptides feature not only hydrogen donor and acceptor capabilities but can also be readily prepared and diversified by conventional methods. Moreover, the penalty of loss of electrostatic interactions between the negatively charged phosphate ions and positively charged ammonium ions of aminoglycosides could be compensated for by introduction of specific basic amino acids. Consequently, efforts directed towards 4,6-peptidyl-2-DOS ligands are described in §6.3.

6.2 Aminoglycoside-type structures

A *de novo* preparation of 4,6-aminosugar-substituted 2-DOS ligands *via* glycosylation requires the preparation of both donor and acceptor molecules. The acceptor molecule, *i.e.* a 5-*O*-protected diazidohexitol deoxystreptamine precursor, was available from earlier efforts, whereas synthesis of the carbohydrate donors was accomplished according to literature procedures. Thus, D-glucosamine was converted into a 2-azidosugar *via* $ZnCl_2$-catalyzed diazotransfer, as described by Wong and co-workers (Scheme 1).[4] Hydroxyls were peracetylated followed by selective deacetylation of the anomeric position with hydrazine acetate to give **2**, and finally conversion into the trichloroacetimidate **3**, which was isolated as a mixture of anomers (α:β, 2:3) in 65% yield.[5]

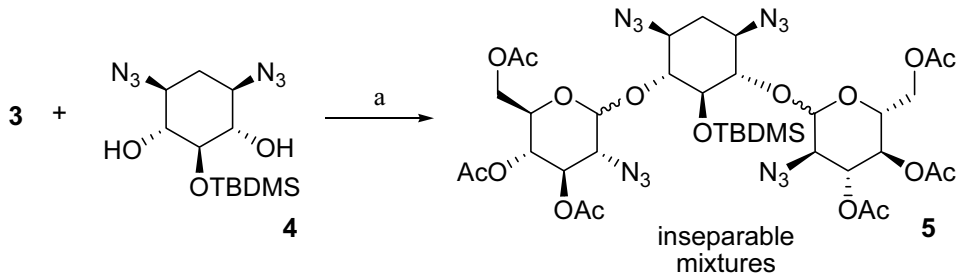
Scheme 1.



Reagents and conditions: (a) TfN_3, Et_3N, $ZnCl_2$, H_2O/MeOH/CH_2Cl_2, 3/10/3, rt, 3 h; (b) Ac_2O, pyridine, rt, 2 h; (c) H_2NNH_2·HOAc, DMF, rt, 2 h; (d) CCl_3CN, K_2CO_3, CH_2Cl_2, rt, 6 h.

Activation of imidate **3** with TMSOTf in the presence of the *O*-TBDMS protected 2-DOS precursor **4** yielded trimeric **5** as a mixture of four diastereoisomers. Unfortunately, separation of the components by column chromatography was elusive (Scheme 2), and consequently analysis was cumbersome.[5] Since the poor selectivity in the glycosidation of 2-azidosugars is typical with most substrates, attention was focused on another carbohydrate donor.

Scheme 2.

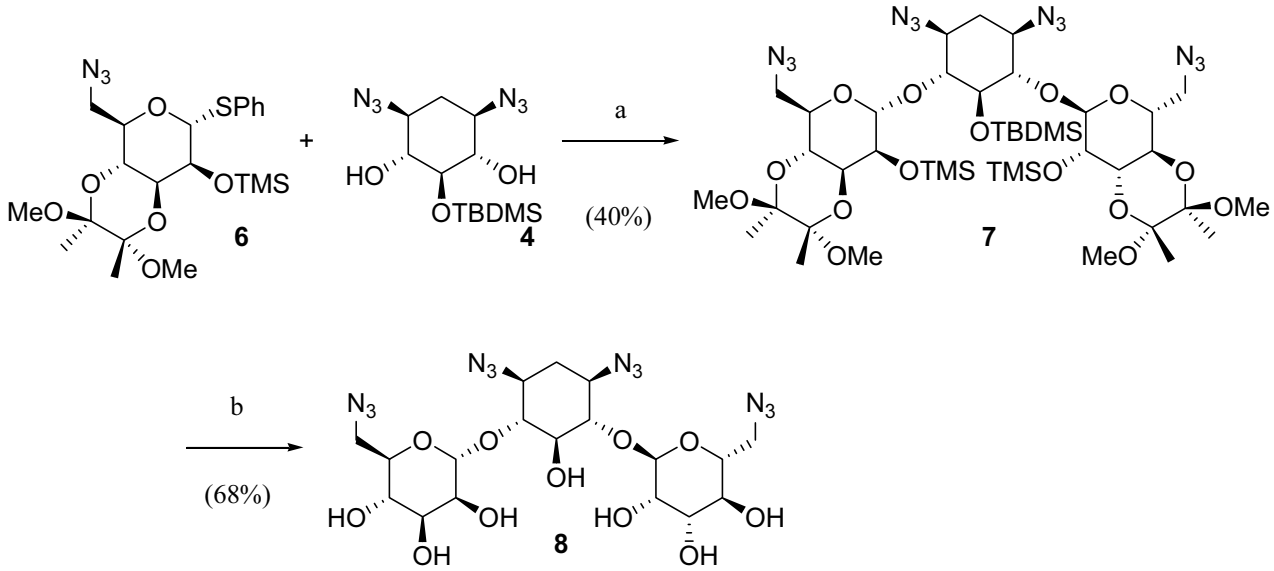


Reagents and conditions: (a) **1**, TMSOTf, 4 Å MS, CH_2Cl_2/Et_2O (1:1), -30 °C, 2h.

Earlier work in our group has established that glycosidation of 6-azidomannothioglycosides under the action of 1-benzenesulfinyl piperidine (BSP) and trifluoromethanesulfonic anhydride (Tf_2O) in the presence of 2,4,6-tri-*tert*-butylpyrimidine (TTBP)[6] generally displays good stereoselectivity and proceeds with high yield.[7] Thus, in adaptation of such a glycosylation strategy,

6-azidomannothioglycoside **6** was activated under the standard conditions followed by addition, and hence attachment, of the TBDMS protected 2-DOS acceptor **4** (Scheme 3). The desired bis-coupled derivative **7** was formed in a yield of 40% with the α,α-coupled product predominating (ratio α,α:β,β:α,β:β,α = 90:4:3:3).[8] The *O*-protective groups were removed in one step by addition of TFA to yield the azido precursor **8** in 68% yield.[9] It was recognized at this stage that the glycodiversification of 2-DOS with an array of donors would lead to a library of purely carbohydrate-based RNA-ligands. Despite the potency of the approach, however, it was also clear that relatively large effort is demanded for the preparation of the required donors and attention was focused on peptidyl-deoxystreptamine conjugates next.

Scheme 3.



Reagents and conditions: (a) BSP, Tf_2O, TTBP, CH_2Cl_2, 4Å MS, -60 ºC, 2h; (b) TFA, 1 min, rt.

6.3 Ester-linked peptidyl 2-DOS

The introduction of peptides onto a 2-DOS scaffold is a versatile means to obtain potential RNA binders because the common set of 20 amino acid building blocks provides the right tools for not only introduction of functionality with great diversity but also for achieving selectivity by combinatorial sequence variation. Our building block **4**, having two free hydroxyls, can be derivatized to a peptidyl-2-DOS conjugate by mono- or bisfunctionalization (symmetrical or asymmetrical), by attachment of the peptide tail(s) to the free alcohols of **4**. We decided to focus on peptides coupled to 2-DOS *via* an ester, an alkyl amide or carbamate functionality (Figure 2).

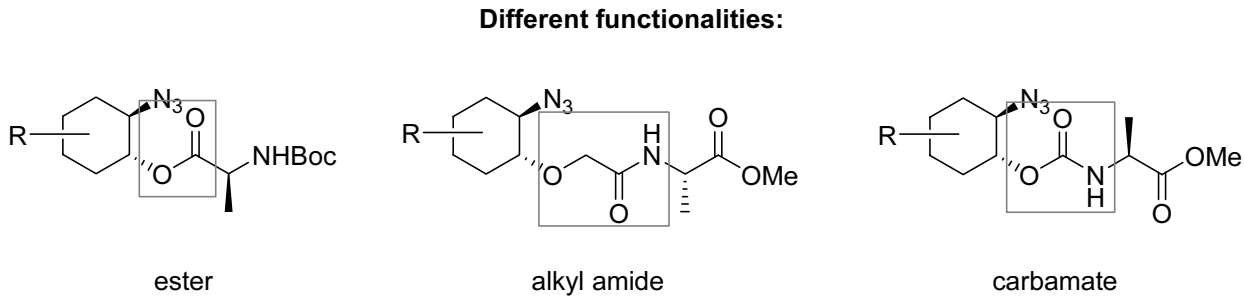
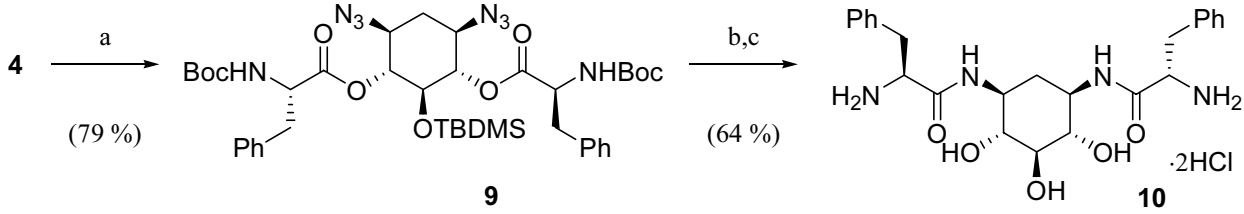


Figure 2.

The most straightforward strategy to couple peptides to 2-DOS scaffold **4** involves attachment of the *C*-terminus *via* an ester. However, before the synthesis of peptidyl-substituted structures was undertaken, we realized that the final product, after reduction of azides to amines, may be prone to *O*→*N* acyl migration. Therefore, in a model study **4** was coupled with *N*-Boc-protected phenylalanine (DCC, DMAP, DCM) under standard esterification conditions to give the bis-amino acyl compound **9** in 79% yield (Scheme 4).

Scheme 4.



Reagents and conditions: (a) Boc-Phe-OH, DCC, DMAP, CH_2Cl_2, 0 °C – rt, 20 h; (b) Pd/C, H_2, 3 bar, MeOH, o.n; (c) 2 M HCl in MeOH, rt, 4 h.

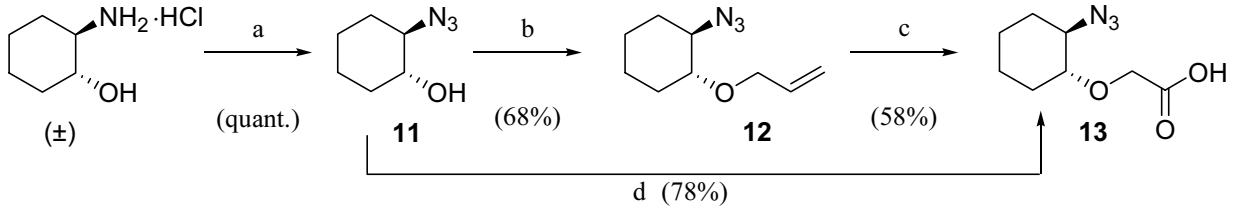
As expected, rearrangement of the amino acyl analogue obtained via hydrogenation of **9** proceeded rapidly and quantitatively, leading to the more stable bis-amide. Subsequent Boc-deprotection under acidic conditions led to an interesting chiral structure (**10**) but the lack of the essential 1,3-diamino functionality of 2-DOS, and hence loss of ionic interactions with the RNA phosphate backbone, gave feed to the expectation that the obtained peptide derivative is a poor RNA binder. Thus, this route was further abandoned and attention was focused on alternative peptidyl linkages.

6.4 Alkylamide-linked peptidyl 2-DOS

Given the fact that direct attachment of peptides through an *O*-acyl linkage turned out unsuitable for our purpose, *O*-alkyl linked derivatives were explored next. However, the lack of variance of amino acid functionalities for direct etherification necessitates the incorporation of a linker (except for Ser en Thr). Azidoalcohol (**11**, Scheme 5), easily synthesized from cheap 1,2-aminocyclohexanol, served as a model system by conversion of the amine, *via* diazotransfer, into azido derivative **11**.[4]

The residual free hydroxyl was allylated followed by oxidation of the alkene[10] leading to acid **13** or, preferably, directly alkylated with bromoacetic acid to yield acid **13** in 78% yield.

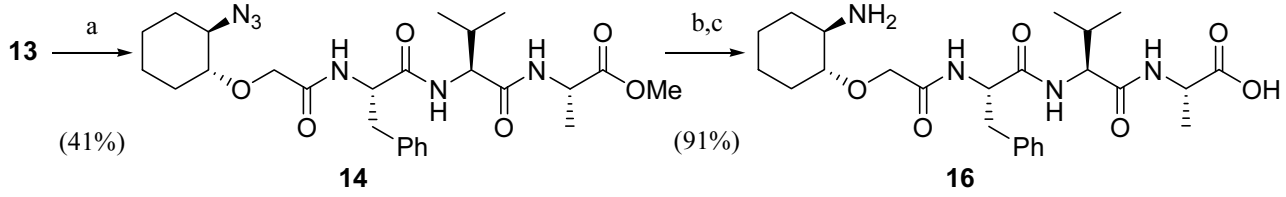
Scheme 5.



Reagents and conditions: (a) TfN_3, Et_3N, $ZnCl_2$, H_2O/MeOH/CH_2Cl_2, 3/10/3, rt, 3 h; (b) AllBr, NaH, TBAI, DMF, 0 °C -rt, 4h; (c) $NaIO_4$, $RuCl_3$, H_2O/$CHCl_3$/MeCN, 2/1/1, rt, 4 ½ h; (d) $BrCH_2CO_2H$, NaH, TBAI, THF, 0 °C - rt, 3 h.

Condensation of acid **13** with the model peptide H-Phe-Val-Ala-OMe, synthesized according to standard Boc-peptide chemistry, yielded the ether-linked peptidyl structure **14** in a yield of 41% (Scheme 6). Subsequent removal of the methyl ester with LiOH followed by reduction of the azides yielded the deprotected model system **16** in high yield (91%).

Scheme 6.

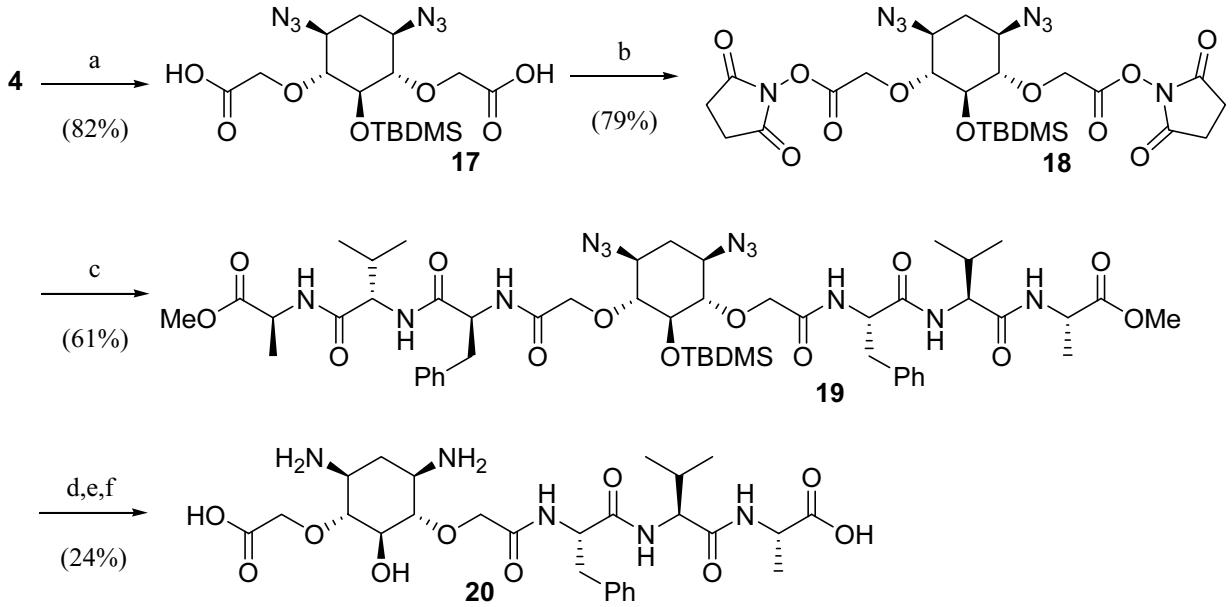


Reagents and conditions: (a) H-Phe-Val-Ala-OMe·HCl (**15**), DIPEA, PyBOP, CH_2Cl_2, rt, o.n.; (b) LiOH, THF/H_2O, 4/1, o.n.; (c) Pd/C, H_2, 3 bar, MeOH, rt, o.n.

The methodology described above was now applied to 2-DOS aminocyclitol **4** by condensation with bromoacetic acid, to yield diacid **17** in 82% yield (Scheme 7). In contrast to above, direct PyBOP-induced coupling of peptide H-Phe-Val-Ala-OMe·HCl (**15**) with diacid **17** failed to produce the desired bis-peptide **19**. Alternatively, conversion of acid **17** into the bis-*N*-hydroxysuccinimide ester (**18**) prior to addition of the tripeptide H-Phe-Val-Ala-OMe·HCl (**15**) was more successful, and led to dual introduction of the tripeptide in a yield of 61%. Deprotection of the peptidyl based ligand was achieved in three steps: (a) hydrogenolysis of azides with Pd/C, (b) removal of the TBDMS protective group and (c) hydrolysis of the methyl ester. Much to our surprise, the resulting end product, isolated in 24% yield for the three steps, was not the expected bis(tripeptide) but mass spectrometric analysis indicated the loss of one of the tripeptide chains. Apparently, the applied conditions for ester hydrolysis were sufficiently basic to induce intramolecular nucleophilic attack of the free 2-DOS alcohol onto the amide bond connecting one of the tripeptides, resulting in

monopeptidyl based ligand **20**. Although such a cleavage can potentially be circumvented by ester hydrolysis prior to TBDMS-removal, the risk of late-stage cleavage of a peptide chain urged us to investigate an alternative strategy.

Scheme 7.



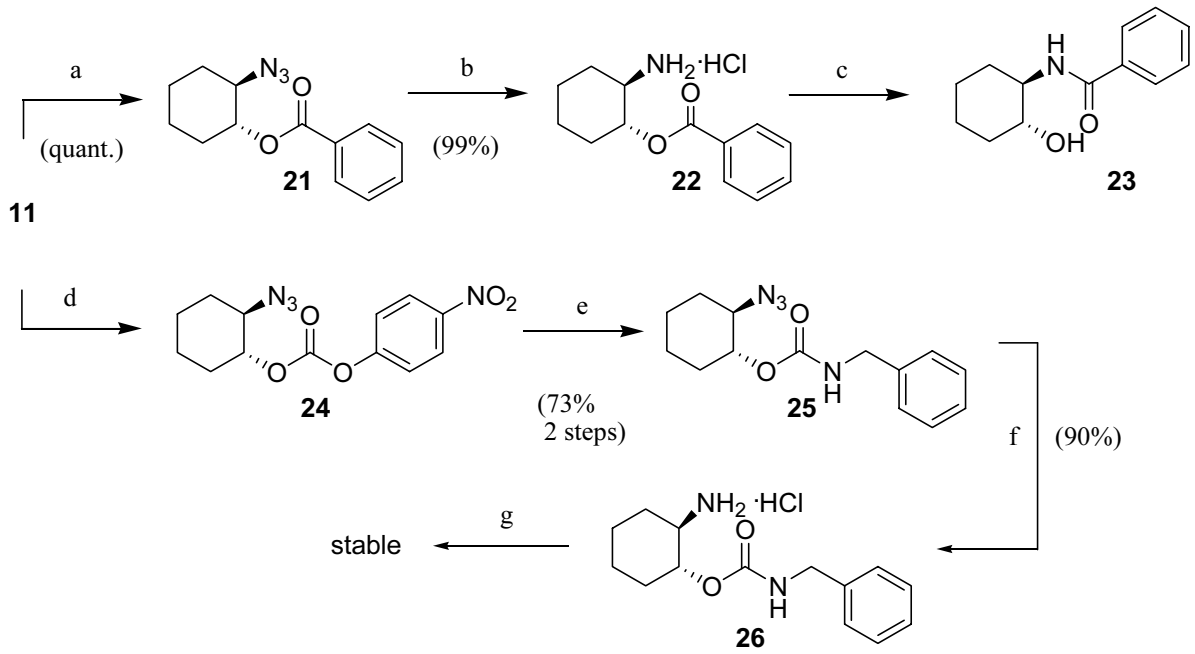
Reagents and conditions: (a) Bromoacetic acid, NaH, TBAI, THF, 0 °C-rt, 3-4h; (b) HOSu, EDC, Et_3N/CH_2Cl_2/DMF (2/5/70), rt, o.n.; (c) H-Phe-Val-Ala-OMe·HCl (**15**), CH_2Cl_2/ DMF/ H_2O (10/5/2), Et_3N, rt, o.n.; (d) $Pd(OH)_2$,[11] H_2, 3 bar, *i*-propanol/H_2O/AcOH (3/2/1), o.n.; (e) 2 M HCl MeOH, rt, 6h; (f) LiOH, THF/H_2O (4/1), rt, o.n.

Earlier reports have indicated that the chance of finding a good peptide-based RNA ligand by random combination of amino acids has a small chance of success. For example, Dardel and co-workers screened a library of hexapeptides against the human tRNAlys3 tRNA by flow-injection NMR, allowing the detection of weak binding interactions. [12] The hexapeptide Tyr-His-Ser-Arg-Asn-Asn (YHSRNN) was found to be structure-specific, and could provide leads for the design of high affinity ligands. In this context, RNA targeting has strong potential since RNA is involved in cellular protein interactions such as transcription, splicing and translation or in viral infection such as human immunodeficiency virus (HIV). Several systematic screens have been preformed on the HIV-1 genomic RNA fragments but in all cases, the high affinity hits were strongly cationic and recognition was dominated by electrostatic effects that were often poorly selective.[13] Thus, there is a need for strategies based on selectivity rather than on affinity. The finding of Dardel *et al.* stimulated us to investigate if the affinity of the strongest binding hexapeptide could be enhanced further by coupling to an RNA-binding pharmacophore such as 2-DOS.

6.5 Carbamate-linked peptidyl 2-DOS

A third alternative linkage of 2-DOS to a peptide is *via* carbamate functionality, which can be easily installed by step-wise condensation of an alcohol and an amine using phosgene-type reagents. However, also in this case an undesired intramolecular *O*→*N* migration leading to a urea, much like the rapid 1,2-rearrangement of esters to a more stable amide bond (*vide supra*), could not be excluded at forehand. To investigate the propensity of such urea formation we prepared the model systems 1,2-aminocarbamate **26** and 1,2-aminoester **22** (Scheme 8). Compound **21** was obtained by reacting (racemic) 2-azidocyclohexan-1-ol (**11**) with benzoyl chloride, followed by hydrogenation in the presence of $CHCl_3$ to give HCl-salt **24**.[14] Carbamate **26** was prepared by consecutive treatment of **11** with 4-nitrophenyl chloroformate and benzylamine and reduction of the azide under aforementioned conditions to afford the corresponding HCl-salt of **26**. To compare the stability of the respective compounds **22** and **26**, the ammonium salts were deprotonated with base, directly followed by NMR analysis. As expected, ester **22** instantaneously rearranged to amide **23**, while carbamate **26** was left unchanged even after heating the reaction mixture at 80 °C for 48 hours, which unequivocally established the usefulness of carbamate functionality for attachment of peptides to 2-DOS.

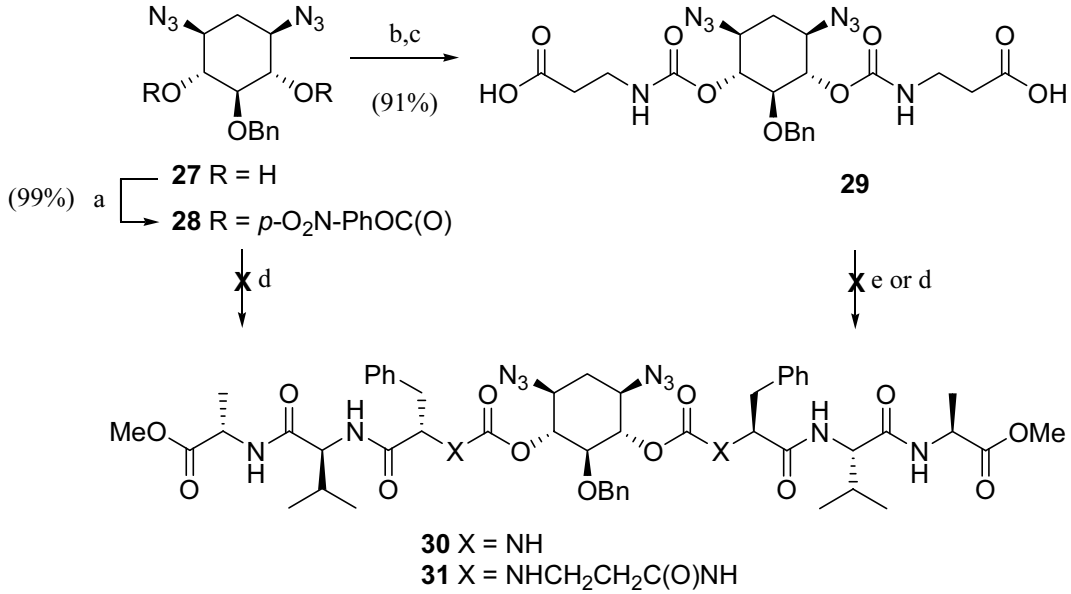
Scheme 8.



Reagents and conditions: (a) BzCl, pyridine, DMAP, CH_2Cl_2, rt, 24h; (b) 10% Pd/C, H_2, EtOH, $CHCl_3$, rt, 3 bar, 24h; (c) DMSO-D_6, aq. 1N NaOH, rt, 4h; (d) 4-nitrophenylchloroformate, pyridine, CH_2CH_2, rt, 2h; (e) $BnNH_2$, DIPEA, CH_2Cl_2, rt, 24h; (f) 10% Pd/C, H_2, EtOH, $CHCl_3$, rt, 2h; (g) CD_3OD, aq. 1N NaOH, 24h.

Thus, in analogy with the model study, 2-DOS precursor **27** was reacted with *p*-nitrophenylchloroformate, leading to the formation of the corresponding activated biscarbonate **28** in high yield (99%, Scheme 9). Rather disappointingly, addition of H-Phe-Val-Ala-OMe to carbonate **28** did not yield the desired biscarbamate **30**, but led to an intractable mixture of components. Since it was rationalized that unfavorable steric clash may have hampered the desired condensation, coupling β-Ala-OMe to the azidocyclitol carbonate (**28**→**29**) prior to peptide addition was investigated. Next, the methyl ester was removed and peptide H-Phe-Val-Ala-OMe was added to the biscarboxylate **29** under peptide coupling conditions (Scheme 9), but again peptidyl based ligand was not formed. Finally, direct coupling of a tetrapeptide **32**, with β-alanine already attached to the *N*-terminus, was also unfortunate since again many products were formed.

Scheme 9.

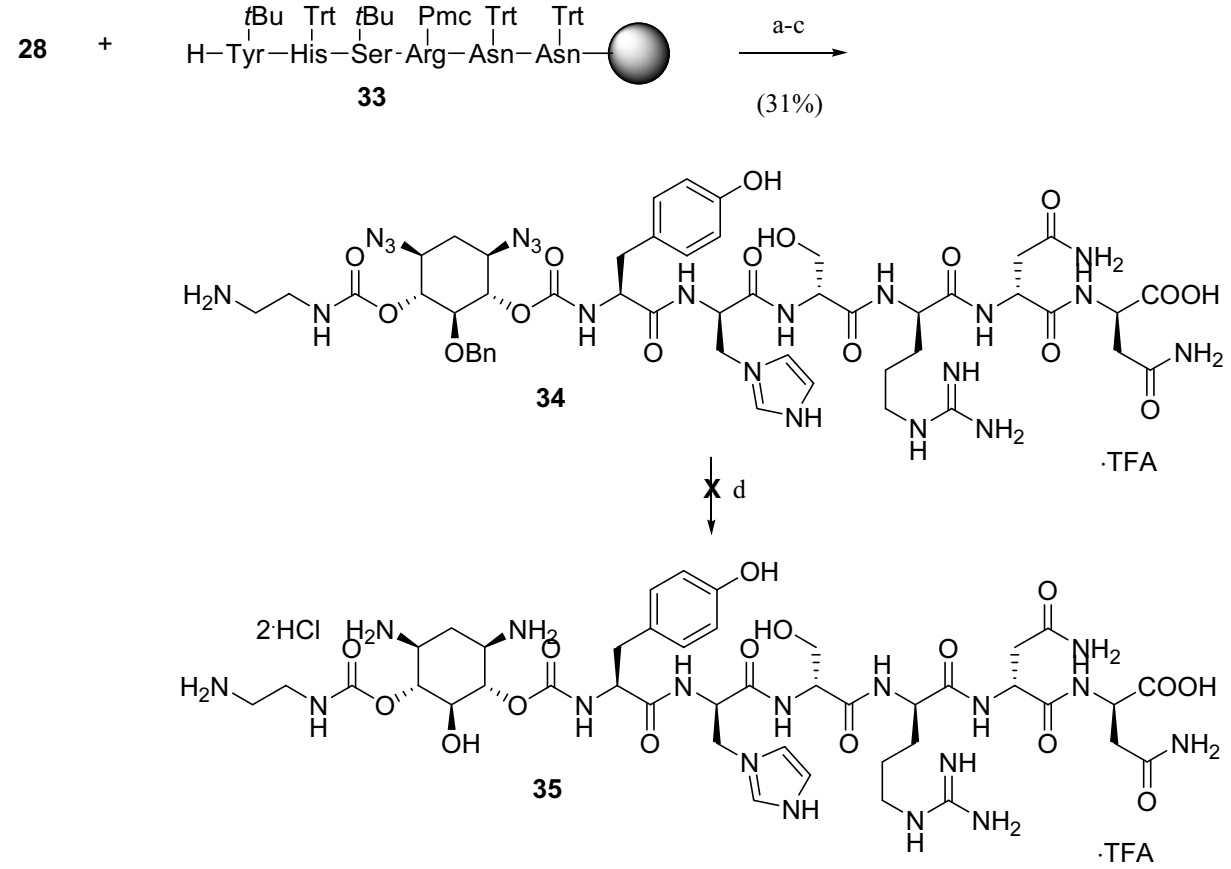


Reagents and conditions: (a) *p*-nitrophenylchloroformate, pyridine, CH_2Cl_2, rt, 4h; (b) β-Ala-OMe·HCl, DIPEA, DMF, rt, o.n.; (c) LiOH, THF/H_2O (4 /1), rt, o.n.; (d) Boc-Phe-Val-Ala-OMe (**15**), TFA, 2h, then BOP, DIPEA,CH_2Cl_2, rt, o.n.; (e) Boc-β-Ala-Phe-Val-Ala-OMe (**32**), TFA, 2h, then BOP, DIPEA,CH_2Cl_2, rt, o.n.

Despite the unfruitful attempts described above it was not excluded that coupling of carbonate **28** may be effected successfully by subjection of a resin-bound peptide to a large excess of **28**. Thus, H-Tyr(*t*-Bu)-His(Trt)-Ser(*t*-Bu)-Arg(Pmc)-Asn(Trt)-Asn(Trt) (**33**), a known tRNA binder as described above, was prepared on Wang resin (loading 0.3 mmol/g) by standard solid phase Fmoc peptide synthesis and reacted with activated carbonate **28** (Scheme 10). Much to our satisfaction, coupling to the hexapeptide proceeded effectively, as indicated by a negative Kaiser test of the resin. Subsequently, excess ethylenediamine was added to quench the second carbonate, followed by cleavage from the resin with simultaneous removal of protective groups, to yield crude peptidyl

2-DOS conjugate **34**. Purification on silica gel gave a homogeneous compound, spectral analysis of which (HRMS, NMR) was in complete agreement with the proposed structure. Unfortunately, deprotection in the final step, *i.e.* azide reduction and benzyl deprotection under a variety of conditions, failed to proceed, and we were hitherto unable to obtain product **35**.

Scheme 10.

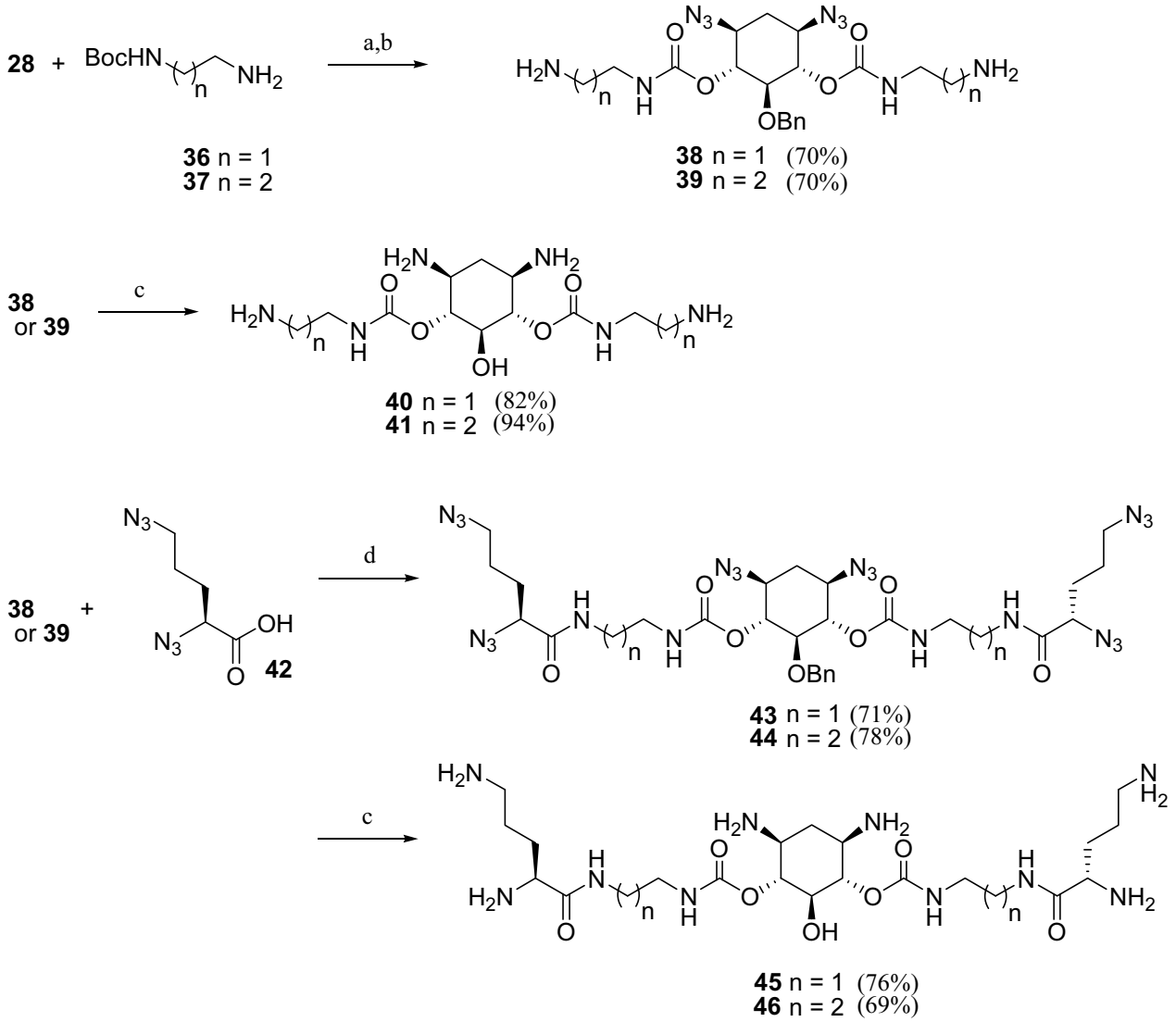


Reagents and conditions: (a) pyridine, HOBt, DMF, 6h.; (b) ethylenediamine, DMF, rt, o.n. (c) TFA/ H_2O/TIS/EDT (180/10/5/5), rt, 2-3h; (d) Pd/C, H_2, EtOH/$CHCl_3$ (5/1) o.n..

At this stage it had become clear that the preparation of 2-DOS peptide conjugates was not as straightforward as at forehand anticipated. However, we realized that interesting 2-DOS based polyamines could also be prepared from intermediate **28** *via* condensation with less intricate structures. For example, condensation of activated carbonate **28** with mono-Boc protected 1,2-ethylenediamine (**36**) or 1,3-propylenediamine (**37**) proceeded smoothly to give, after deprotection of the Boc group a free diamine (Scheme 11). Apart from being a direct precursor of a tetraamine derivative of 2-DOS, by virtue of the presence of the free amine functionality, amino acids can be directly *C*-coupled to **38** and **39**. For example, coupling of the bisazido derivative of ornithine to either ethylene- or propyleneamine extended structures **38** or **39** yielded the respective peptidyl ligands **43** and **44** in 71% and 78% yield. Several conditions to reduce the azides and to

remove the benzyl were investigated such as Raney-nickel, Pd/C in several solvents, and PMe_3 in THF, and it was determined that optimal conditions were found to involve simultaneous hydrogenolysis of azides and removal of the benzyl group under the action of $Pd(OH)_2$ in an *i*-propyl alcohol/H_2O/AcOH mixture to yield compound **45** and **46** in 76% and 69% yield, respectively.

Scheme 11.



Reagents and conditions: (a) DIPEA, DMF, rt, o.n.; (b) TFA/CH_2Cl_2 (1/1), rt, 2h; (c) $Pd(OH)_2$, H_2, 3 bar, *i*-propanol/ H_2O/AcOH (3/2/1), o.n. (d) BOP, DIPEA, HOBt, rt, o.n.

6.6 Concluding remarks

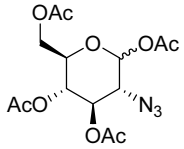
Previously synthesized diazidocyclitols were established as useful scaffolds for the preparation of 2-DOS based carbohydrate and peptide conjugates. For example, functionalized 6-azidomannothioglycoside (**6**) was coupled successfully to 2-DOS to form trimeric aminoglycoside **7** in 40% yield and with high selectivity. Conjugation of peptides to 2-DOS *via* ester functionality was found unsuitable for the preparation of new RNA binders, due to rapid

O→*N* migration. On the other hand, alkylamide and carbamate functionalized 2-DOS ligands were found sufficiently stable for preparation of new peptidyl 2-DOS derivatives, although care has to be taken in application of basic conditions to avoid peptide cleavage by intramolecular amide hydrolysis. The protected hexapeptide H-Tyr(*t*-Bu)-His(Trt)-Ser(*t*-Bu)-Arg(Pmc)-Asn(Trt)-Asn(Trt) was successfully coupled to 2-DOS but deprotection was not successful sofar. The structures **20** and **40**, **41**, **45**, **46** resulting from the synthetic efforts described above are currently under evaluation as potential RNA affinity ligands by flow-through NMR detection of binding to a tRNA construct.

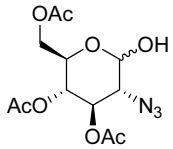
6.7 Acknowledgements

B. Ritzen and B. Verheijen are gratefully acknowledged for their synthetic contributions to this chapter. D. Gironés is kindly acknowledged for the glycosylation of 2-DOS with the thioglycoside. Dr. D. Löwik and H. Adams are gratefully acknowledged for preparing the hexapeptide and fruitful discussions.

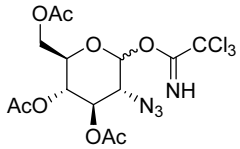
6.8 Experimental Section



2-Azido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-α,β-glucopyranoside (1)
Glucosamine·HCl (4.00 gr, 4.63 mmol) and $ZnCl_2$ (6 mg, 1 mol%) were dissolved in water (15.5 mL). Et_3N (1.93 mL, 13.9 mmol) and, slowly, MeOH (50 mL) were added. TfN_3 in CH_2Cl_2 (0.6 M, 15.5 mL) was added at once to the vigorously stirring solution. The reaction mixture was stirred for 3 hours at room temperature and quenched with $NaHCO_3$ (sat. aq.) the solvents were evaporated. The crude product was now dissolve in pyridine (25 mL, 185 mmol) and Ac_2O (15 mL, 62 mmol) and DMAP (cat.) were added. After stirring the reaction mixture for 2 hours at rt, H_2O was added and the product was extracted with EtOAc, dried with $MgSO_4$ and the solvent were evaporated. Flash column chromatography (EtOAc/*n*-heptane, 3/2) to yield **1** (1.21 g, 69%) as a white solid. R_f 0.44 (EtOAc/*n*-heptane, 1/1). IR υ_{max} film: (cm^{-1}) 2941, 2111, 1748, 1363, 1208, 1035, 611. ^1H NMR ($CDCl_3$, 300 MHz, ppm): 5.53 (d, J = 8.4 Hz, 1H), 5.08-4.98 (m, 2H), 4.28 (dd, J = 4.5, 12.0 Hz, 1H), 4.11-3.99 (m, 2H), 3.78-3.68 (m, 1H), 2.18 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 170.3, 169.4, 168.3, 92.6, 90.1, 72.9, 72.8, 70.9, 69.9, 67.9, 62.8, 61.6, 60.5, 21.1. In agreement with literature.[15]

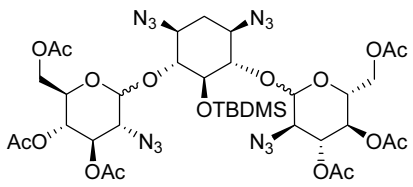


2-Azido-2-deoxy-3,4,6-tri-*O*-acetyl-α,β-glucopyranoside (2)
Compound **1** (100 mg, 0.268 mmol) was dissolved in dry DMF (0.5 mL) and H_2NNH_2·HOAc (25 mg, 0.27 mmol) were added. The reaction mixture was stirred for 2 hours. Quench with *i*PrOH/EtOAc (1/9) and wash with water. The product was purified with flash column chromatography (EtOAc/*n*-heptane, 1/1) and compound **2** was obtained as colorless oil (75.4 mg, 85%). R_f 0.35 (EtOAc/*n*-heptane, 1/1). IR υ_{max} film: (cm^{-1}) 3442, 2950, 2360, 2110, 1745, 1432, 1367, 1225, 1140, 1044, 962, 911, 734. ^1H NMR ($CDCl_3$, 400 MHz, ppm): 5.53 (t, J=9.2 Hz, 1H), 5.07-4.94 (m, 2H), 4.31-4.19 (m, 2H), 4.18-4.08 (m, 1H), 3.52-3.39 (m, 1H), 2.08 (s, 9H). HRMS (ESI) *m/z* calcd for $C_{12}H_{18}O_8N_3$ $(M+H)^+$: 332.1094, found 332.1097.[15]



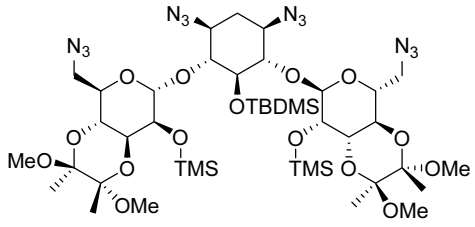
2-Azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-trichloroimidate-α,β-glucopyranoside (3)
Compound **2** (118 mg, 0.36 mmol) and CCl_3CN (80 µL, 0.80 mmol) were dissolved in dry CH_2Cl_2 (2 mL). Freshly powdered K_2CO_3 (44 mg, 0.32 mmol) was added and the

reaction was stirred for 6 hours. Filter over Celite and the product was purified with flash column chromatography (EtOAc/*n*-heptane, 1/2) and compound **3** (110 mg, 65%) was obtained as a white solid. R_f 0.20 (β), 0.27 (α) (EtOAc/*n*-heptane, 1/2). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.84 (s, 1H), 6.50 (s, 1H), 5.52 (t, *J* = 9.6 Hz, 1H), 5.16 (t, *J* = 9.6 Hz, 1H), 4.40-4.28 (m, 2H), 4.13-4.07 (m, 1H), 3.78 (dd, *J* = 3.5, 10.4 Hz, 1H), 2.10 (s, 9H). In agreement with literature.[15]



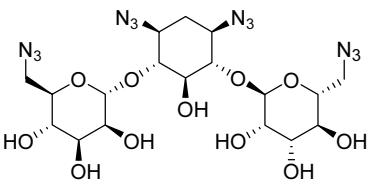
4,6-Diazido-2-[(*tert*-butyldimethylsilyl)oxy]cyclohexanediol-(1',2)-(1''-6)-di(2-azido-2-deoxy-3,4,6-tri-*O*-acetylglucopyranoside) (5)

Compound **4** (30 mg, 0.091 mmol) and **3** (65 mg, 0.14 mmol) were coevaporated three times with toluene. 4 Å MS and a mixture of CH_2Cl_2/Et_2O (1/1, 6 mL) were added to compound **4**. The reaction mixture was cooled to -30 °C and TMSOTf (40 µL, 0.22 mmol) was added slowly. The reaction was stirred for 2 hours and quenched with DIPEA (40 µL, 0.22 mmol). After the reaction was diluted with CH_2Cl_2 (12 mL) the mixture was allowed to warm up to room temperature. The reaction mixture was washed with 0.1 M HCl, dried with $MgSO_4$ and the solvent was evaporated to yield an inseparable mixture of diastereoisomers (**5**).



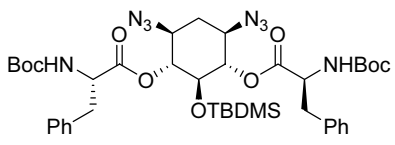
4,6-Diazido-2-[(*tert*-butyldimethylsilyl)oxy]cyclohexanediol-(1'-2)-(1''-6)-di(6-desoxy-azido-2-trimethylsilyl-3,4-dimethoxybutane-manopyranoside) (7)

Compound **4** and the thioglycoside **6** were coevaporated three times with toluene. To the stirred solution containing activated 4 Å MS (80 mg) the thioglycoside **6** (122 mg, 0.252 mmol), 1-benzenesulphinyl (BSP, 63 mg, 0.30 mmol) and TTBP (156 mg, 0.63 mmol) in CH_2Cl_2 (5 mL) at -60 °C was added Tf_2O (51 µL, 0.30 mmol). After stirring the reaction mixture was for 10 min. 2-DOS acceptor **4** (41 mg, 0.126 mmol) was added in CH_2Cl_2 (5 mL). After stirring the reaction for 20 minutes the reaction mixture was quenched with $P(OEt)_3$ (52 µL, 0.30 mmol) and warmed up to -30 °C. Saturated aq. $NaHCO_3$ was added and the reaction was warmed up to rt after which the reaction mixture was extracted with CH_2Cl_2, dried with Na_2SO_4 and evaporated. The product was purified by flash column chromatography (EtOAc/*n*-heptane, 1/8) to obtain **7** (54 mg, 40%) as a white solid. R_f 0.4 (EtOAc/*n*-heptane, 2/3). $[\alpha]_D^{20}$ + 114 (c 0.25; CH_2Cl_2). IR υ_{max} film: cm^{-1} 2950, 2098, 1128, 1038, 842, 779. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.70 (d, 1H, *J* = 3.2 Hz, Th), 7.32-7.69 (m, 3H, arom), 6.79 (d, 2H, *J* = 8.5 Hz, arom), 5.50 (m, 1H, allyl), 5.14-4.89 (m, 3H, allyl and CH), 4.35 (d, 1H, *J* = 14.9 Hz, CH_2), 3.79 (s, 4H, OMe and CH), 3.51 (d, 1H, *J* = 14.9 Hz, CH_2), 2.78 (m, 1H, CH_2a), 2.36(m, 1H, CH_2b), 1.44 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 159.3, 158.3, 142.9, 134.7, 130.0, 119.0, 118.4, 114.2, 82.0, 74.7, 65.4, 55.9, 55.0, 36.1, 32.6, 29.7, 23.44, 14.89. HRMS (ESI) *m/z* calcd for $C_{42}H_{78}O_{15}N_{12}Si_3Na$ $(M+Na)^+$ 1097.4915, found: 1097.4925.



4,6-Diazido-2-[(*tert*-butyldimethylsilyl)oxy]cyclohexanediol-(1'-2)-(1''-6)-di(6-desoxy-azido-2-manopyranoside) (8)

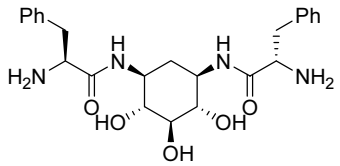
Compound **7** (35 mg, 0.032 mmol) was dissolved in 1 mL TFA. The mixture was stirred for 1 min and evaporated. The product was purified with flash column chromatography (EtOAc to H_2O/MeCN, 1/4) to obtain **8** (19 mg, 68%) as a white solid. IR υ_{max} film: cm^{-1} 3367, 2103, 1672, 1259, 1132, 1051, 839. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.41 (br s, 1H), 5.15 (br s, 1H), 4.23-4.08 (m, 2H), 3.99 (br s, 1H), 3.79-3.51 (m, 7H), 3.50-3.90 (m, 7H), 2.42-2.22 (m, 1H), 1.61-1.53 (m, 1H). ^{13}C NMR($CDCl_3$, 100 MHz, ppm): δ 102.8, 102.6, 83.8, 81.5, 76.5, 74.1, 73.7, 72.2, 72.1, 71.9, 71.9, 69.2, 68.8, 62.2, 60.7, 53.0, 52.7. HRMS (FAB) *m/z* calcd for $C_{18}H_{28}O_{11}N_{12}$ $(M+H)^+$: 589.2079, found: 589.2088; $(M+Na)^+$ 611.1899 found: 611.1877.



1,3-diazido-5-*O*-(*tert*-butyldimethylsilyl)- 4,6-bis-*O*-[(2*S*)-2-(*tert*-butyloxycarbonyl)amino-3-phenylpropanoyl]-1,3-dideamino-2-deoxystreptamine (9)

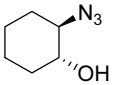
N-Boc-L-PheOH (145 mg, 0.546 mmol) was dissolved in CH_2Cl_2 and cooled to 0 °C. DCC (122 mg, 0.546 mmol) and DMAP (67 mg, 0.456

mmol) were added and the reaction was stirred at 0 °C for 20 minutes. Compound **4** (60 mg, 0.182 mmol) was added and the reaction was stirred at 0 °C for 4 hours. The reaction mixture was filtered after stirring for 20 hours at room temperature. CH_2Cl_2 was evaporated, and the residue was dissolved in EtOAc, washed once with $NaHCO_3$ (sat), H_2O and NaCl (sat). The solvents were evaporated and the product was purified with flash column chromatography (EtOAc/*n*-heptane, 1/10) to yield **9** (118 mg, 79%) as colorless oil. IR υ_{max} film: (cm^{-1}) 2929, 2099, 1704, 1365, 1162, 536. ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 7.31-7.04 (m, 10H), 5.12-4.84 (m, 2H), 5.03-4.72 (m, 2H), 4.68-4.49 (m, 2H), 3.70 (s, 4H), 3.54-3.29 (m, 2H), 3.12-2.98 (m, 1H), 1.99-1.78 (m, 1H), 1.56 (s, 18H), 1.38 (s, 9H), 0.92-0.61 (m, 1H), 0.13 (s, 3H), 0.08 (s, 3H). HRMS (FAB) *m/z* calcd for $C_{40}H_{59}O_9SiN_8$ $(M+H)^+$: 823.4174, found: 823.4181.



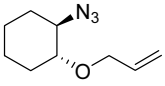
***N,N'*-[(2*S*)-2-amino-3-phenylpropanoyl]-2-deoxystreptamine (10)**

Compound **9** (92 mg, 0.11 mmol) was dissolved in MeOH (8.0 mL) and Pd/C (10%, 8.0 mg) was added. After stirring at room temperature for 19 h under an atmosphere of H_2, the mixture was filtered off and the filtrate was concentrated *in vacuo* affording the crude product (55 mg, 64%). To a solution of the obtained material (29 mg, 38 μmol) in MeOH (2.0 mL) at 0 °C was slowly added AcCl (0.14 ml, 2.0 mmol). The mixture was allowed to rise to room temperature and stirred for 16 h. Upon completion, the reaction mixture was concentrated *in vacuo* and the crude product was purified using an Isolute SPE column (Flash SCX-2 resin) affording the product **10** (17 mg, quant) as an off-white solid. IR υ_{max} film: (cm^{-1}) 3283, 2922, 1643, 1553, 1097, 1030. ^1H NMR (DMSO-D6, 400 MHz, ppm): δ 7.72-7.69 (m, 2H, NH), 7.28-7.16 (m, 10H, arom), 3.47-2.83 (m, 9H, CH and CH_2), 2.60-2.50 (m, 2H, CH_2), 1.84-1.72 (m, 1H, CH_2a), 1.09-0.96 (m, 1H, CH_2b). ^{13}C NMR (CD_3OD, 75 MHz, ppm): δ 175.9, 138.5, 130.1, 129.3, 127.5, 77.7, 76.2, 52.3, 51.4, 42.5, 35.3.



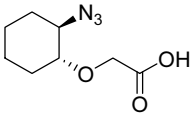
(±)-(1*R*,2*R*)-2-azidocyclohexanol (11)

To a solution of (±)-(1*R*, 2*R*)-2-aminocyclohexanol (3.4 g, 22 mmol), $ZnCl_2$ (31 mg, 1 mol%), and Et_3N (9.4 mL, 67 mmol) in H_2O (56 mL) was slowly added MeOH (185 mL). TfN_3 in CH_2Cl_2 (0.6 M, 56 mL) was added at once to the vigorously stirring solution and the reaction mixture was stirred for 3 hours at room temperature. Upon completion, the mixture was quenched with saturated aqueous $NaHCO_3$ and the solvent evaporated *in vacuo*. The crude product was purified using colmn chromatography (EtOAc/*n*-heptane, 1/5) to obtain the desired product **11** (3.2 g, quant) as a colourless oil. R_f 0.35 (EtOAc/*n*-heptane, 1/5). IR ν_{max} film: (cm^{-1}) 3350, 2937, 2862, 2095. ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 3.42-3.34 (m, 1H, CH), 3.21-3.13 (m, 1H, CH), 2.09-2.00 (m, 2H, CH_2), 1.79-1.74 (m, 2H, CH_2), 1.41-1.23 (m, 4H, CH_2).



(±)-(1*R*,2*R*)-1-(allyloxy)-2-azidocyclohexane (12)

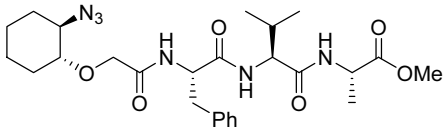
A solution of azidocyclohexanol **11** (50 mg, 0.35 mmol) in DMF (4.0 mL) was cooled to 0 °C and TBAI (2 mg, 1 mol%), NaH (36 mg, 0.91 mmol) and allylbromide (45 μL, 0.53 mmol) were added. After stirring for 3 h at room temperature, the mixture was quenched using saturated aqueous NH_4Cl and *i*-PrOH/EtOAc (1/9) was added. The mixture was extracted three times with H_2O, dried with Na_2SO_4 and concentrated *in vacuo* to yield the crude product. Purification by column chromatography (EtOAc/*n*-heptane, 1/10) resulted in isolation of **12** as a colourless (for UK) oil (43 mg, 68%). R_f 0.67 (EtOAc/*n*-heptane, 1/5). ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.92 (tdd, 1H, J = 5.6, 10.4, 17.2 Hz), 5.31 (ddd, 1H, J = 1.6, 3.4 Hz, 17.2 Hz), 5.14 (ddt, 1H, J = 1.3, 1.8, 10.4 Hz), 4.11 (ddt, 1H, J = 1.5, 5.7, 12.7 Hz), 4.02 (ddt, 1H, J = 1.6, 5.5, 12.6 Hz), 3.30-3.23 (m, 1H), 3.17-3.12 (m, 1H), 2.09-2.03 (m, 1H), 1.95-1.89 (m,1H), 1.70-1.63 (m, 2H), 1.24-1.16 (m, 4H).



(±)-2-[(1*R*,2*R*)-2-azidocyclohexyloxy]acetic acid (13)

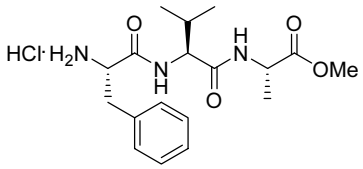
NaH (63 mg, 1.6 mmol) was washed three times with *n*-heptane and dissolved in THF (1.0 mL). The mixture was cooled to 0 °C and a solution of bromoacetic acid (80 mg, 0.58 mmol) and TBAI (6.0 mg, 1 mol%) in THF (1.0 mL) was added dropwise. After stirring for 1 h, azidocyclohexanol **11** (50 mg, 0.35 mmol) was added and the mixture was allowed to warm to room temperature and stirred for 3 h. The reaction mixture was diluted with EtOAc and quenched with 10% aqueous HCl solution. The aqueous solution was extracted three times with EtOAc. The combined organic organic layers were dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column

chromatography (EtOAc and 2% AcOH) to obtain the product **13** (55 mg, 78%, based on ^{1}H NMR) and bromoacetic acid as an inseparable mixture. R_f 0.54 (EtOAc and 2% AcOH). IR ν_{max} film: (cm^{-1}) 2937, 2863, 2095, 1446, 1258, 1116. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.22 (s, 2H), 3.39-3.33 (m, 1H), 3.28-3.22 (m, 1H), 2.16-2.2.10 (m, 1H), 2.01-1.94 (m, 1H), 1.74-1.67 (m, 2H), 1.36-1.22 (m, 4H). ^{13}C NMR (CD_3OD, 75 MHz, ppm): δ 173.9, 83.4, 67.5, 65.9, 31.3, 31.2, 24.8, 24.5. HRMS (CI) *m/z* calcd for $C_8H_{14}N_3O_3$ $(M+H)^+$: 200.1038, found: 200.1035.



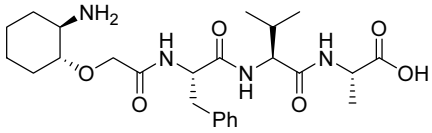
Methyl *N*-{[(1*R*,2*R*)-2-azidocyclohexyloxy]acetyl} phenylalaninylvalinylalaninate (14)

A solution of HCl·H-Phe-Val-Ala-OMe (35 mg, 91 μmol) and DIPEA (35 μL, 0.20 mmol) in CH_2Cl_2 (3.0 mL) were stirred for 5 min at room temperature. Azidocyclohexanol **13** (27 mg, 0.10 mmol) and PyBOP (52 mg, 0.10 mmol) were added and the reaction mixture was stirred for 24 h at room temperature. After removal of the solvent the crude product was purified by column chromatography (EtOAc) which afforded **14** (20 mg, 41%) as an off-white solid. R_f 0.41 (EtOAc and 2% AcOH). IR ν_{max} film: (cm^{-1}) 2937, 2863, 2097, 1736, 1647, 1451, 1358, 1260, 1235, 845. ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 7.28-7.18 (m, 5H, arom), 4.42-4.36 (m, 1H), 4.24-4.20 (m, 3H, CH and CH_2), 4.03-4.00 (m, 1H), 3.70 (s, 3H, OMe), 3.27-3.11 (m, 2H), 3.04-2.94 (m, 2H), 2.13-1.69 (m, 3H, CH and CH_2), 1.71-1.65 (m, 2H), 1.40 (d, 3H, J = 7.9 Hz), 1.31-1.23 (m, 4H), 0.96 (dd, 6H, J = 6.8, 13.0 Hz). ^{13}C NMR (CD_3OD, 75 MHz, ppm): δ 173.9, 172.6, 172.4, 172.2, 130.1, 129.2, 128.0, 127.0, 83.4, 69.1, 65.8, 59.8, 55.3, 52.7, 49.5, 39.2, 32.4, 31.3, 25.0, 24.7, 19.7, 18.9, 17.4. HRMS (CI) *m/z* calcd for $C_{26}H_{39}N_6O_6$ $(M+H)^+$: 531.2931, found: 531.2931.



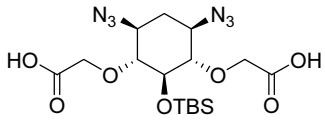
HCl·NH_2-Phe-Val-Ala-OMe (15)

According to the general peptide coupling procedure, to a solution of HCl·H-Val-Ala-OMe (2.56 g, 10.7 mmol) and DIPEA (4.10 mL, 23.6 mmol) in CH_2Cl_2 (360 mL), were added Boc-Phe-OH (3.13 g, 11.8 mmol) and PyBOP (12.3 g, 23.6 mmol) and the reaction mixture was stirred for 24 h. Purification by column chromatography (MeOH/CH_2Cl_2, 1/9) afforded the product (4.81 g, quant) as a white solid. R_f 0.42 (MeOH/CH_2Cl_2, 1/9). ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 7.28-7.17 (m, 5H, arom), 4.40-4.30 (m, 2H, CH), 4.22 (dd, 1H, J = 7.4, 8.6 Hz, CH), 3.70 (s, 3H, OMe), 3.10 (dd, 1H, J = 5.3, 13.9 Hz, CH_2), 2.81 (dd, 1H, J = 9.3, 13.8 Hz, CH_2), 2.05 (dq, 1H, J = 6.9, 13.8, 20.6 Hz, CH), 1.40-1.35 (m, 12H, *t*-Bu and CH_3), 0.97 (dd, 6H, J = 6.8, 12.2 Hz, CH_3). HRMS (FAB) *m/z* calcd for $C_{23}H_{36}N_3O_6$ $(M+H)^+$: 450.2604, found: 450.2605. According to the general Boc-deprotection procedure, a solution of Boc-Val-Ala-OMe (3.25 g, 10.7 mmol) in 2 M HCl/EtOAc (110 mL) was stirred for 3 h. Work-up afforded the HCl-salt **15** (2.56 g, quant) as a white solid. ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 8.35 (d, 1H, J = 6.7 Hz, NH), 6.51 (d, 1H, J = 8.6 Hz, NH), 4.46-4.38 (m, 1H, CH), 3.92-3.88 (m, 1H, CH), 3.70 (s, 3H, OMe), 2.02 (dq, 1H, J = 6.7, 13.5, 20.2 Hz, CH), 1.39 (d, 3H, J = 7.3 Hz, Me), 0.95 (dd, 1H, J = 6.8, 20.8 Hz, Me).



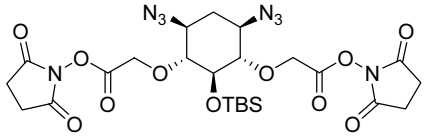
***N*-{[(1*R*,2*R*)-2-aminocyclohexyloxy]acetyl} phenylalaninylvalinylalanine and *N*-{[(1*S*,2*S*)-2-aminocyclohexyloxy]acetyl}phenylalaninylvalinylalanine (16)**

Compound **14** (28 mg, 52 μmol) was dissolved in H_2O/THF (mL, 1/4). LiOH (4 mg, 0.16 mmol) was added, and the mixture was stirred overnight at room temperature. THF was evaporated *in vacuo*, and the aqueous phase was acidified with 10% aqueous HCl solution and extracted three times with EtOAc. The combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo*. The crude product (27 mg, 52 μmol) which was used without further purification was redissolved in MeOH (5.0 mL) and Pd/C (10%, 5 mg) was added. After stirring at room temperature for 19 h, the mixture was filtered off and the filtrate was concentrated *in vacuo*. The crude product was purified using an Isolute SPE column (Flash SCX-2 resin) affording the product **16** (32 mg, 91%) as an off-white solid. R_f 0.14 (H_2O/MeCN, 1/4). IR ν_{max} film: (cm^{-1}) 3374, 2929, 2856, 1740, 1649, 1535, 1449, 1221, 1123. ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 7.29-7.18 (m, 5H, arom), 4.24-4.00 (m, 5H), 3.30-3.10 (m, 2H), 3.03-2.84 (m, 2H), 2.22-2.01 (m, 3H), 1.82-1.72 (m, 2H), 1.45-1.33 (m, 7H), 0.99-0.94 (m, 6H). ^{13}C NMR (CD_3OD, 75 MHz, ppm): δ 175.2, 173.6, 172.2, 171.9, 130.2, 129.1, 127.6, 127.2, 83.4, 69.0, 68.7, 67.5, 65.7, 55.2, 54.8, 38.9, 33.1,32.4, 31.2, 25.0, 24.6, 18.8. HRMS (ESI) *m/z* calcd for $C_{25}H_{39}N_4O_6$ $(M+H)^+$: 491.2870, found: 491.2831.



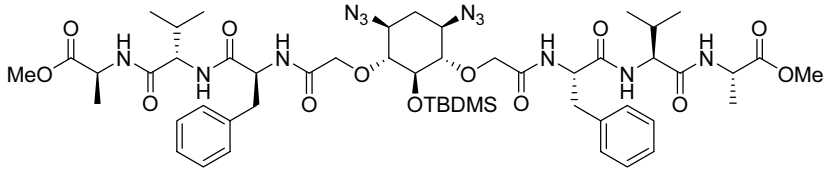
2,2'-[1,3-diazido-5-*O*-(*tert*-butyldimethylsilyl)-1,3-dideamino-2-deoxystreptamine-4,6-*O*-diyl]diacetic acid (17)

NaH (83 mg, 2.1 mmol) was washed three times with *n*-heptane and dissolved in THF (1.0 mL). The mixture was cooled to 0 °C and a solution of bromoacetic acid (0.11 mg, 0.76 mmol) and (1 mg, 1 mol%) TBAI in THF (1.0 mL) was added dropwise. After stirring for 1 h, azidocyclohexanol **4** (75 mg, 0.23 mmol) was added and the mixture was allowed to warm to room temperature and stirred for 3 h. The reaction mixture was diluted with EtOAc and quenched with 10% aqueous HCl solution. The aqueous solution was extracted three times with EtOAc. The combined organic organic layers were dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc and 2% AcOH to MeOH/$CHCl_3$, 1/9) to obtain the product **17** (84 mg, 82%). R_f 0.17 (MeOH/$CHCl_3$, 1/9). IR ν_{max} film: (cm^{-1}) 3365, 2954, 2872, 2358, 2101, 1635, 1254, 1087 836. ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 4.48-4.30 (m, 4H, CH_2), 3.62-3.53 (m, 1H, CH), 3.43-3.23 (m, 4H, CH), 2.19 (dt, 1H, J = 4.1, 12.7 Hz CH_2a), 1.36 (dd, 1H, J = 12.4, 24.9 Hz, CH_2b), 0.93 (s, 9H, *t*-Bu), 0.18 (s, 3H, Me), 0.10 (s, 3H, Me). ^{13}C NMR (CD_3OD, 75 MHz, ppm): δ 174.4, 85.9, 77.2, 61.3, 33.0, 26.5, 18.8, -4.0, -4.5. HRMS (ESI) *m/z* calcd for $C_{16}H_{28}N_6O_7Si$ $(M+Na)^+$: 467.1686, found: 467.1729.



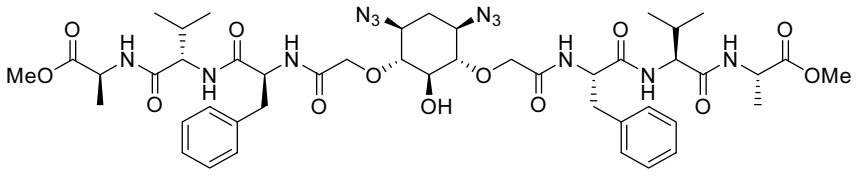
***N*-{2,2'-[1,3-azido-5-*O*-(*tert*-butyldimethylsilyl)-1,3-dideamino-2-deoxystreptamine-4,6-*O*-diyl]bisacetoxy}disuccinimide (18)**

To a solution of **17** (245 mg, 0.550 mmol), *N*-hydroxysuccinimide (130 mg, 1.13 mmol), Et_3N (32 µL), and DMF (100 µL) in CH_2Cl_2 (1.2 mL) was added EDC (217 mg, 1.13 mmol) at 0 °C. The mixture was stirred at room temperature for 19 h and evaporated *in vacuo*. The residue was dissolved in EtOAc and washed with H_2O. The organic layer was dried with $MgSO_4$ and evaporated *in vacuo* to obtain **17** (277 mg, 79%) as a white solid. IR ν_{max} film: (cm^{-1}) 2927, 2848, 2104, 1737, 1258, 1203, 1084. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.82-4.64 (m, 4H), 3.55-3.48 (m, 1H), 3.40-3.22 (m, 4H), 2.82 (s, 8H), 2.25 (dt, 1H, J = 4.4, 13.1 Hz), 1.40 (dd, 1H, J = 12.8, 25.6 Hz), 0.92 (s, 9H, *t*-Bu), 0.17 (s, 3H, Me), 0.11 (s, 3H, Me). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 165.1, 164.8, 85.6, 76.0, 68.2, 62.0, 32.3, 30.0, 18.4, -3.9, -4.3. HRMS (CI) *m/z* calcd for $C_{24}H_{34}N_8O_{11}SiNa$ $(M+Na)^+$: 661.2014, found: 661.2054.



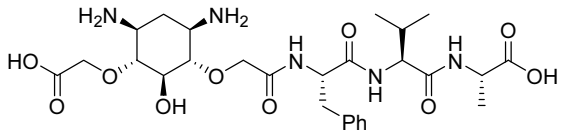
Dimethyl *N,N'*-{2,2'-[1,3-azido-5-*O*-(*tert*-butyldimethylsilyl)-1,3-dideamino-2-deoxystreptamine-4,6-*O*-diyl]bisacetoxy}di(phenyl alaninylvalinylalaninate) (19)

To a suspension of HCl·H-Phe-Val-Ala-OMe (63 mg, 0.16 mmol) in DMF (1.5 mL), H_2O (0.6 mL), Et_3N (30 µL), and CH_2Cl_2 (3.0 mL) at 0 °C was added **18** (50 g, 78 µmol). The mixture was stirred for 1 h at 0 °C, and the temperature was allowed to rise to room temperature within 19 h. The solvent was removed *in vacuo*, and the residue was dissolved in EtOAc/*n*-heptane and washed with H_2O three times. The organic layers were combined, dried with $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (EtOAc/*n*-heptane, 1/1 to H_2O/MeCN, 1/4) to obtain **19** (53 mg, 61%). R_f 0.65 (H_2O/MeCN, 1/4). IR ν_{max} film: (cm^{-1}) 3300, 2933, 2868, 2366, 2327, 2102, 1740, 1648, 1539, 1256, 1090. ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 7.28-7.14 (m, 10H, arom), 4.45-4.35 (m, 2H), 4.23-4.07 (m, 8H), 3.71 (s, 6H, OMe), 3.37-3.32 (m, 1H), 3.20-3.09 (m, 6H), 3.01-2.91 (m, 2H), 2.26-2.17 (m, 1H), 2.08-1.97 (m, 2H), 1.47-1.38 (m, 7H), 1.00-0.91 (m, 17H), 0.18 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (CD_3OD, 75 MHz, ppm): δ 173.1, 173.0, 172.6, 172.4, 171.0, 138.3, 138.0, 137.9, 130.6, 130.5, 130.4, 129.5, 127.9, 127.8, 84.8, 84.6, 77.5, 77.4, 73.4, 72.3, 63.3, 61.2, 59.9, 55.3, 55.0, 54.9, 54.7, 52.7, 52.6, 39.4, 39.3, 39.0, 32.8, 32.2, 26.5, 19.1, 18.9, 17.4, -3.5, -4.3. $[\alpha]_D^{20}$ = -28.4 (c = 0.61, H_2O). HRMS (ESI) *m/z* calcd for $C_{52}H_{78}N_{12}O_{13}SiNa$ $(M+Na)^+$: 1129.5478 found: 1129.53996.



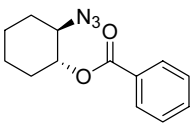
Dimethyl *N,N'*-[2,2'-(1,3-azido-1,3-dideamino-2-deoxystreptamine -4,6-*O*-diyl)bisacetoxy] di(phenylalaninylvalinylalaninate)

A solution of **19** (30 mg, 27 µmol) in 2 M HCl/EtOAc (2.0 mL) was stirred for the 2-3 h at room temperature. The reaction mixture was diluted with EtOAc and quenched with H_2O solution. The aqueous layer was extracted three times with EtOAc. The combined organic organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. To afford the crude product (27 mg, quant) as a white solid which was used without further purification. ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 7.32-7.17 (m, 10 H, arom), 4.43-4.17 (m, 10H), 3.70 (s, 6H, OMe), 3.62-3.51 (m, 1H), 3.41-3.25 (m, 3H), 3.22-3.08 (m, 3H), 3.04-2.89 (m, 2H), 2.18-2.09 (m, 3H), 1.41-1.25 (m, 7H), 0.99-0.94 (m, 6H). HRMS (ESI) *m/z* found for $C_{46}H_{65}N_{12}O_{13}$ $(M+H)^+$: 993.



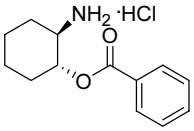
2-[4-*O*-(*N*-acetoxyphenylalaninylvalinylalaninyl)-2-deoxystreptamin-6-*O*-yl]acetic acid (20)

A solution of the compound described above (27 mg, 27 µmol) and LiOH (4.0 mg, 0.16 mmol) in H_2O/THF (2.0 mL, 1/4) was stirred for 16 h. After stirring for 18 h at room temperature THF was removed *in vacuo*. EtOAc was added and the H_2O solution was acidified using 10 % aqueous hydrochloric acid. The aqueous layer was extracted with EtOAc four times, dried with Na_2SO_4 and concentrated *in vacuo*. To a solution of the above-obtained material in *i*PrOH/H_2O/AcOH (3/1/1, 10 mL) was added charcoal (25 mg). After brief stirring, the charcoal was filtered off and washed with *i*PrOH. The filtrate was concentrated *in vacuo* and redissolved in *i*PrOH/H_2O/AcOH (3/1/1, 10 mL). Pd/C (25 mg about, 1time the weight of the compound) was added and the mixture was placed under an atmosphere of H_2 and stirred overnight. The Pd/C was filtered off and the filtrate was concentrated *in vacuo*. The Pd/C was filtered off and the filtrate was concentrated *in vacuo*. The crude product was purified by prep-TLC to yield product **20** (3.9 mg, 24%). R_f 0.56 (25% NH_3 in H_2O/$CHCl_3$/*n*-BuOH/EtOH, 6/2/4/5). ^{1}H NMR (H_2O, 400 MHz, ppm): δ 7.29-7.13 (m, 5H, arom), 4.35-3.80 (m, 7H), 3.68-3.11 (m, 5H), 3.04-2.91 (m, 2H), 2.31-2.26 (m, 1H, CH_2a), 2.11-2.04 (m, 1H), 1.67-1.63 (m, 1H, CH_2b), 1.22-1.18 (m, 3H, Me), 0.81-0.77 (m, 6H, Me). (ESI) *m/z* found for $C_{27}H_{42}N_5O_{10}$ $(M+H)^+$: 596.



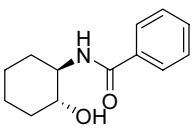
(±)-(1*R*, 2*R*)-2-azido-1-benzoicacidcyclohexanol (21)

To a solution of compound **11** (100 mg, 0.718 mmol) in CH_2Cl_2 (7mL) was added pyridine (87 µL, 1.07 mmol) and benzoylchloride (125 µL, 1.07 mmol). After 2 h DMAP (cat.) was added the reaction mixture was stirred overnight and washed with a 2N solution of citric acid, brine and dried with Na_2SO_4. The solvent was evaporated and the product was purified with flash column chromatography (EtOAc/*n*-heptane, 1/10) to yield **21** (176 mg, quant.) as a white powder. R_f 0.43 (EtOAc/*n*-heptane, 1/5), IR υ_{max} film: (cm^{-1}) 2094, 1728, 1268, 1108, 710. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.98-8.11 7.49-7.58 (m, 2H, arom), 7.35-7.46 (m, 2H, arom), 5.01-4.82 (m, 1H, CH), 3.68-3.48 (m, 1H, CH), 2.28-2.15 (m, 1H, CH_2), 2.13-1.96 (m, 1H, CH_2), 1.86-1.18 (m, 2H, CH_2), 1.28-1.1.49 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 165.9, 133.2, 129.8, 128.5, 76.1, 63.6, 30.6, 30.5, 23.9, 23.6. HRMS (CI) *m/z* calcd MS $C_{13}H_{16}O_2N_3$ $(M+H)^+$: 246.1245, found: 246.1247.



(±)-(1*R*, 2*R*)-2-amino hydrochloride-1-benzoicacidcyclohexanol (22)

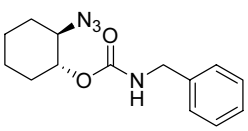
Compound **21** (80 mg, 0.33 mmol) was dissolved in EtOH (25 mL) and $CHCl_3$ (0.5 mL). 10 % Pd/C (50 mg) was added to the solution and the reaction was shaken overnight under 3 Barr hydrogen atmosphere in a Parr apparatus. The reaction mixture was filtered trough Hyflo-Supercel and evaporated to yield **22** (82 mg, 99%.) as a cream colored powder. IR υ_{max} film: (cm^{-1}) 2929, 1714, 1262, 710. ^{1}H NMR (DMSO-D_6, 400 MHz, ppm): δ 8.44 (br s, 3H, NH), 8.06 (dd, 2H, *J* = 1.3, 8.4 Hz, arom), 7.55 (t, *J* = 7.4 Hz, 1H, arom), 7.53 (t, *J*= 7.7 Hz, 2H, arom), 4.88 (dt, *J* = 4.5, 10.3 Hz, 1H, CH), 3.09-2.92 (m, 1H, CH), 2.22-2.03 (m, 2H, CH_2), 1.82-1.61 (m, 2H, CH_2), 1.52-1.31 (m, 4H, CH_2). ^{13}C NMR (D_2O, 100 MHz, ppm): δ 167.4, 133.6, 129.1, 128.2, 74.5, 52.7, 44.1, 29.4, 28.5, 22.6, 22.5, 21.7, 21.0. HRMS (CI) *m/z* calcd for $C_{13}H_{18}NO_2$ $(M+H)^+$: 220.1338, found: 220.1344.



(±)-(1*R*, 2*R*)-2-aminobenzoicacidcyclohexanol (23)

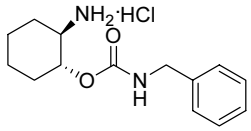
Compound **22** was dissolved in DMSO –D6 and a 1 N solution of NaOH was added. The reaction was followed by NMR. NMR of the rearranged product **23**: ^{1}H NMR (DMSO-D_6, 400 MHz, ppm): δ 7.98-7.78 (m, 2H, arom), 7.39-7.28 (m, 3H, arom), 2.9-2.85 (m, 1H, CH), 2.28-2.07 (m, 1H, CH), 1.70-1.69 (m, 2H, CH_2), 1.61-1.49 (m, 2H, CH_2), 1.1.44-1.38

(m, 1H, CH_2), 1.18-0.93 (m, 3H, CH_2). ^{13}C NMR (D_2O, 75 MHz, ppm): δ170.1, 139.9, 128.9, 127.0, 75.1, 56.3, 46.5, 33.6, 32.9, 27.2, 26.4, 24.4, -0.7.



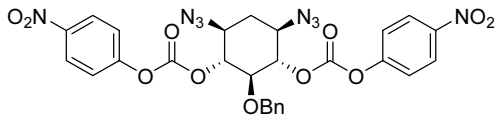
(±)-(1*R*, 2*R*)-Benzylcarbamicacid-2-azidocyclohexylester (25)

To a solution of compound **11** (100 mg, 0.718 mmol) in CH_2Cl_2 (7mL) was added pyridine (87 µL, 1.07 mmol) and 4-nitrophenylchloroformate (217 mg, 1.07 mmol). After stirring for 2h the reaction mixture was washed with a 2N solution of citric acid, brine and dried with Na_2SO_4. Evaporate the organic solvent and dissolve the reaction mixture in CH_2Cl_2 and add heptane, the white solid was filtered of and the organic solvents were evaporated the procedure was repeated three times. R_f 0.61 (EtOAc/*n*-heptane, 3/2). The obtained compound was again dissolved in CH_2Cl_2 (7 mL) and benzylamine (80 µL, 0.93 mmol) and DIPEA (162 µL, 0.93 mmol) were added. After the reaction mixture had been stirred overnight the reaction mixture was diluted with CH_2Cl_2 and washed with $NaHCO_3$ (sat.) and dried with Na_2SO_4. The solvent was evaporated and the product was purified with flash column chromatography (EtOAc/*n*-heptane, 1/10, to 2/1) to yield **25** (144 mg, 73%) as a white powder. R_f 0.18 (EtOAc/*n*-heptane, 1/5), IR υ_{max} film: (cm^{-1}) 3315, 2938, 2095, 1701, 1258, 611. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.40-7.21 (m, 5H, arom), 5.12 (br s, 1H, NH), 4.52-4.78 (m, 1H, CH), 4.38 (d, 2H, J = 5.9 Hz, CH_2), 3.42-3.21 (m, 1H, CH), 2.18-2.11 (m, 1H, CH_2), 2.07-1.94 (m, 1H, CH_2), 1.69-1.60 (m, 2H, CH_2) 1.40-1.20 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 155.8, 138.5, 128.8, 127.6, 76.4, 63.7, 45.2, 31.2, 30.4, 23.9, 23.6. HRMS (CI) *m/z* calcd for $C_{14}H_{19}N_4O_2(M+H)^{+:}$ 275.1508, found: 275.1510.



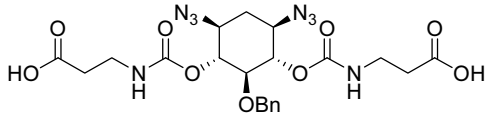
(±)-(1*R*,2*R*)-Benzylcarbamicacid-2-aminohydrochloridecyclohexylester (26)

See procedure of compound (**22**). The hydrogenation was carried out at atmospheric pressure; according to TLC the reaction was finished after 2h. IR υ_{max} film: (cm^{-1}) 2937, 1701, 1526, 1453, 1252, 700. ^1H NMR (CD_3D, 400 MHz, ppm): δ 7.52-7.12 (m, 5H, arom), 4.56 (br s, 1H, NH), 4.29-4.19 (m, 2H, CH_2), 3.72-3.49 (m, 1H, CH), 3.29-3.09 (m, 1H, CH), 2.29-2.03 (m, 2H, CH_2), 1.79-1.56 (m, 2H, CH_2), 1.58-1.09 (m, 4H, CH_2). ^{13}C NMR (CD_3OD, 75 MHz, ppm): δ157.8, 140.2, 129.5, 128.2, 75.6, 58.3, 54.7, 45.6, 31.9, 30.4, 24.7, 18.4. HRMS (CI) *m/z* calcd for $C_{14}H_{20}O_2N_2$ $(M+H)^+$: 249.1603, found: 249.1609.



1,3-diazido-5-*O*-benzyl-1,3-dideamino-2-deoxy-4,6-*O*-bis[(4-nitrofenoxy)carbonyl]streptamine (28)

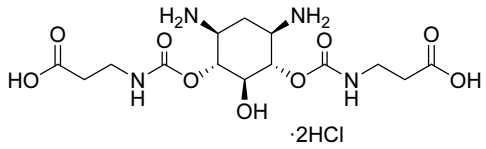
To a solution of compound **27** (40 mg, 0.13 mmol) in CH_2Cl_2 (1.5 mL) were added 4-nitrophenylchloroformate (80 mg, 0.40 mmol) and pyridine (32 µL,0.39 mmol). After stirring for 3 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 and 10% aqueous citric acid solution was added. The aqueous layer was extracted with EtOAc three times and the combined layers were washed with H_2O and brine, dried with Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc/*n*-heptane, 1/5 to 1/3 to 100% EtOAc) to afford **28** (82 mg, 99%). R_f 0.28 (EtOAc/*n*-heptane, 1/3). IR υ_{max} film: cm^{-1} 3123, 2859, 2102, 1775, 1525, 1348, 1251, 1210, 860. ^1H NMR (Aceton-D6, 400 MHz, ppm): δ 8.32 (d, 4H, J = 9.3 Hz, arom), 7.42 (d, 4H, J = 9.3 Hz, arom), 7.37-7.35 (m, 5H, arom), 5.22 (t, 2H, J = 9.8 Hz, CH), 4.88 (s, 2H, CH_2 OBn), 4.13 (t, 1H, J = 9.6 Hz, CH), 4.09-4.02 (m, 2H, CH), 2.59 (dt, 1H, J = 4.7, 12.8 Hz, CH_2a), 2.04 (dd, 1H, J = 12.4, 25.1 Hz, CH_2b). ^{13}C NMR (Aceton-D6, 75 MHz, ppm): δ 156.6, 139.3, 129.8, 129.1, 128.8, 126.7, 123.5, 82.0, 80.9, 76.7, 59.4, 32.2. HRMS (ESI) *m/z* calcd for $C_{27}H_{22}N_8O_{11}Na$ $(M+Na)^+$: 657.1306 found: 657.1353.



***N,N'*-[(1,3-diazido-5-*O*-benzyl-1,3-dideamino-2-deoxystreptamin-4,6-*O*-diyl)dicarbonyl]bis(*β*-alanine) (29)**

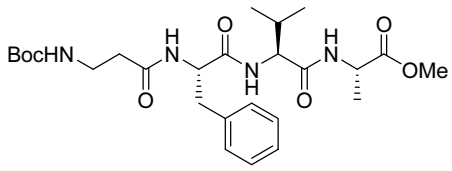
To a solution of **28** (40 mg, 63 µmol) in DMF (2.0 mL) were added *β*-H-Ala-OMe (26 mg, 0.19 mmol) and DIPEA (44 µl, 0.25 mmol). After stirring for 21 h at room temperature, the mixture was concentrated *in vacuo* and covaporated with toluene. After addition of *i*-PrOH/EtOAc (1/9), the mixture was extracted with H_2O four times, dried with Na_2SO_4 and concentrated *in vacuo*. Column chromatography (EtOAc/*n*-heptane, 1/1 to MeOH/$CHCl_3$, 1/9) afforded the compound (32 mg, 91%) as a white solid. R_f 0.79 (MeOH/$CHCl_3$, 1/9). IR υ_{max} film: cm^{-1} 3287, 3049, 2950, 2928, 2101, 1737, 1697, 1531, 1251, 1013. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.33-7.22 (m, 5H, arom), 5.26 (bs, 2H, NH), 4.91 (t, 2H, J = 9.9 Hz, CH), 4.59 (s, 2H, CH_2 OBn),

3.65 (s, 6H, OMe), 3.53-3.37 (m, 7H, CH and CH_2), 2.52 (ddq, 4H, J = 4.7, 6.9, 17.2 Hz, CH_2), 2.22 (dt, 1H, J = 4.5, 13.2 Hz, CH_2a), 1.51 (dd, 1H, J = 13.3, 26.4 Hz, CH_2b). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 173.1, 155.0, 137.9, 127.9, 127.8, 126.3, 79.5, 76.2, 74.3, 58.8, 51.9, 36.8, 34.1, 31.9. HRMS (CI) *m/z* calcd for $C_{23}H_{31}N_8O_9$ $(M+H)^+$: 563.2214, found: 563.2203.To a solution of the compound (31 mg, 55 µmol) in H_2O/THF (1.0 mL, 1/4) was now added LiOH (8.0 mg, 0.33 mmol). After stirring for 16 h at room temperature the reaction mixture was concentrated *in vacuo*. After removal of the solvent, the product was redissolved in H_2O and acidified using aqueous hydrochloric acid (1M). The aqueous solution was extracted with EtOAc four times, dried with Na_2SO_4 and concentrated *in vacuo* to afford **29** (30 mg, quant) as a white solid. R_f 0.47 (H_2O/MeCN, 1/4). ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 7.23-7.13 (m, 5H, arom), 4.74 (t, 2H, J = 9.9, CH), 4.52 (s, 2H, CH_2 OBn), 3.52 (ddd, 2H, J = 7.1, 14.1, 20.3 Hz, CH), 3.27 (t, 4H, J = 6.6 Hz, CH_2), 2.40 (t, 4H, J = 6.6 Hz, CH_2), 2.10 (dt, 1H, J = 3.9, 8.4 Hz, CH_2a), 1.35 (dd, 1H, J = 12.6, 25.2 Hz, CH_2b).



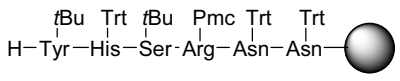
_N,N'_-[(2-deoxystreptamin-4,6-_O_-diyl)dicarbonyl]bis(_β_-alanine) dihydrochloride

According to general procedure for the deprotection, to a solution of **29** (7.0 mg, 13 µmol) in *i*-PrOH/H_2O/AcOH (8.0 mL) was added $Pd(OH)_2$ (14 mg). Work-up and purification afforded the product (5.9 mg, 98%) as an off-white solid. R_f 0.35 (25% NH_3 in H_2O/$CHCl_3$/*n*-BuOH/EtOH, 6/2/4/5). IR υ_{max} film: cm^{-1} 3131, 3038, 2353, 1718, 1527, 1400, 1242, 1077. ^{1}H NMR (D_2O, 400 MHz, ppm): δ 4.89 (t, 2H, J = 10.0 Hz, CH), 3.79 (t, 1H, J = 9.6 Hz, CH), 3.66-3.59 (m, 2H, CH), 3.47-3.35 (m, 4H, CH_2), 2.58 (dt, 1H, J = 4.0, 8.5 Hz, CH_2a), 2.52-2.48 (m, 4H, CH_2), 2.10-1.98 (m, 7H, Me AcOH and CH_2b). ^{13}C NMR (D_2O, 75 MHz, ppm): δ 178.8, 156.4, 73.4, 71.0, 48.4, 47.7, 37.0, 35.5, 21.2. HRMS (ESI) *m/z* calcd for $C_{14}H_{24}N_4O_9$ $(M+H)^+$: 393.



Boc-_β_-Ala-Phe-Val-Ala-OMe (32)

According to the general peptide coupling procedure, to a solution of TFA·H-Phe-Val-Ala-OMe (424 mg, 1.10 mmol) and DIPEA (422 µL, 2.42 mmol) in CH_2Cl_2 (20.0 mL), were added Boc-*β*-Ala-OH (229 mg, 1.21 mmol) and PyBOP (630 mg, 1.21 mmol) and the reaction mixture was stirred for 48 h. Purification by column chromatography (EtOAc/*n*-heptane, 2/1) afforded **32** (228 mg, 40%). R_f 0.24 (EtOAc/*n*-heptane, 2/1). ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 7.24-7.14 (m, 5H, arom), 7.09 (bm, 1H, NH), 7.01 (bm, 1H, NH), 6.89 (bm, 1H, NH), 5.28 (bs, 1H, NH t-Bu), 4.73 (q, 1H, J = 7.2, 14.1 Hz, CH), 4.55-4.48 (m, 1H, CH), 4.27 (t, 1H, J = 7.8 Hz, CH), 3.70 (s, 3H, OMe), 3.35 (t, 2H, J = 6.8 Hz, CH_2), 3.08 (dd, 1H, J = 6.1, 13.9 Hz, CH_2), 2.96 (dd, 1H, J = 7.8, 14.0 Hz, CH_2), 2.42 (t, 2H, J = 5.7 Hz, CH_2), 2.03 (dq, 1H, J = 6.9, 13.8, 20.6 Hz, CH), 1.39-1.36 (m, 12H, *t*-Bu and CH_3), 0.88 (dd, 6H, J = 6.8, 14.2 Hz, CH_3).

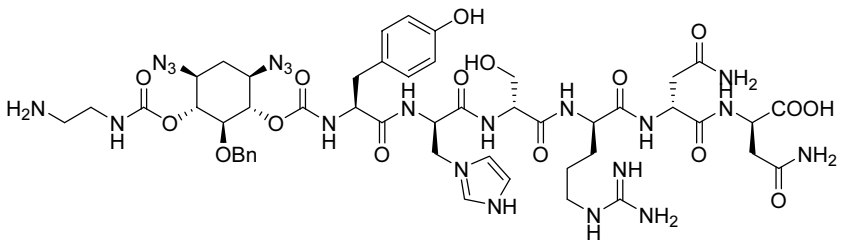


Fmoc-Asn(Trt)-Asn(Trt)-Arg(Pmc)-Ser(*t*-Bu)-His(Trt)-Tyr(*t*-Bu)-OH (33)

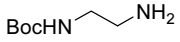
A suspension of Wang-resin (5.94 g, 1.136 mmol/g OH) in DMF (60.0 mL) was cooled to 0 °C after which Fmoc-Asn(Trt)-OH (8.00 g, 13.5 mmol), HOBt (3.65 g, 27.0 mmol) and DIPCDI (1.70 g, 13.5 mmol) was added. This mixture was shaken for 6 h. The functionalised resin was filtered and washed repeatedly with CH_2Cl_2, DMF and *i*-PrOH. Unfunctionalised groups on the resin were capped by adding benzoylchloride (2.04 mL, 17.6 mmol) and pyridine (1.68 mL, 20.6 mmol) to a suspension of the resin in CH_2Cl_2 (60.0 mL) at 0 °C. This mixture was shaken for 30 minutes, filtered and washed repeatedly with CH_2Cl_2, DMF and *i*-PrOH. Then the Fmoc-Asn(Trt)-OH functionalised resin (loading 0.3 mmol/g) was swollen and filtered three times in DMF (50.0 mL). Next DMF (50.0 mL) containing 20 v/v % piperidine (3 * 6 min) was added to remove the Fmoc group. A positive Kaiser test indicated completeness of this reaction. The next amino acid was coupled by adding a mixture of Fmoc-Asn(Trt)-OH (24.1 g, 40.5 mmol 3eq), in DMF (180 mL), with DIPCDI (5.63 g, 22.3 mmol 3.3 eq.) and HOBt (6.57 g, 48.6 mmol 3.6 eq). A negative Kaiser test indicated completeness of the reaction after which the mixture was washed with DMF and twice with CH_2Cl_2 and *i*-PrOH and again with DMF. This procedure was repeated with the following four amino acids: Fmoc-Arg(Pmc)-OH, Fmoc-Ser(*t*-Bu)-OH, Fmoc-His(Trt)-OH, Fmoc-Tyr(*t*-

Bu)-OH. HRMS (FAB) *m/z* calcd for $C_{47}H_{58}N_{13}O_{13}$ $(M+H)^+$: 1012.4277, found: 1012.4282 (cleavage from resin with TFA/H_2O/TIS/EDT, 180/10/5/5, 3 h).

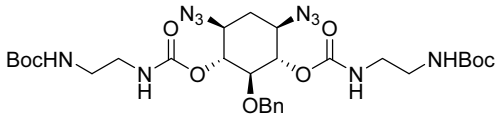
General procedure for peptide couplings reactions in solution A solution of amino ester and DIPEA (2.2 equiv) in CH_2Cl_2 was stirred for 5 min at room temperature under Ar-atmosphere. Boc-protected amino acid (1.1 equiv) and PyBOP (1.1 equiv) were added and the reaction mixture was stirred at room temperature for the indicated time. After removal of the solvent the crude product was purified with column chromatography.



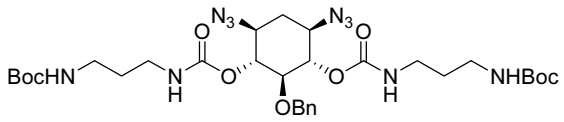
***N*-({6-*O*-[(2-aminoethyl) carbamoyl]-1,3-diazido-5-*O*-benzyl-1,3-dideamino-2-deoxystreptamin-4-*O*-yl}carbonyl)tyrosinylhistidinylserinylarganinylarganinylaspargine trifluoroacetic acid salt (34)** Functionalised Wang resin **33** (0.10 g, 0.20 mmol) was swollen in DMF for 30 minutes. After which pyridine (40 µL, 0.49 mmol), HOBt (0.14 g, 1.0 mmol) and **28** (50 mg, 79 µmol) were added. After shaking for 6 h, ethylenediamine (21 µL, 0.32 mmol) was added and the resulting mixture was shaken for 13 h. Then the resin was filtered and washed with DMF, CH_2Cl_2, *i*-PrOH, CH_2Cl_2 and Et_2O. After the resin was dried, TFA/H_2O/TIS/EDT (4.0 mL, 180/10/5/5) was added and the resin was shaken for 2 h. The mixture was concentrated *in vacuo* and the desired product **34** (30 mg, 31%) was precipitated in Et_2O and freeze dried from H_2O to yield a fluffy white solid. IR υ_{max} film: cm^{-1} 3326, 2359, 2339, 2104, 1669, 1516, 1254, 1203, 1136. ^1H NMR (D_2O, 400 MHz, ppm): δ 8.64-8.59 (m, 1H), 8.27-8.26 (m, 1H), 7.39-6.64 (m, 11H), 4.76-4.18 (m, 15H), 3.95-3.67 (m, 4H), 3.55-2.69 (m, 10H), 2.41-2.29 (m, 1H), 1.93-1.49 (m, 5H). ^{13}C NMR (D_2O, 75 MHz, ppm): δ 175.4, 174.8, 173.5, 172.2, 172.1, 163.7, 163.2, 157.3, 155.0, 140.9, 137.7, 134.1, 131.0, 130.9, 129.2, 119.0, 117.8, 116.1, 115.2, 80.3, 78.0, 77.2, 76.2, 62.0, 58.8, 58.6, 57.6, 56.4, 54.4, 54.3, 51.4, 41.5, 40.1, 39.0, 37.6, 37.5, 37.3, 31.6, 29.2, 27.5, 25.4. HRMS (ESI) *m/z* calcd for $C_{49}H_{68}N_{21}O_{16}$ $(M+H)^+$ 1206.5153 found: 1206.5121.



***N-tert*-Butoxycarbonyl-1,2-ethanediamine (36)** A solution of $(Boc)_2O$ (5.00 g, 23.9 mmol) in dioxane (60 mL) was added over a period of 2.5 h to a solution of 1,2-diaminoethane (10.8 g, 181 mmol) in dioxane (60 mL). The mixture was allowed to stir for 24 h and the solvent was evaporated *in vacuo*. H_2O (100 mL) was added to the residue and the insoluble bis-substituted product was removed by filtration. The filtrate was extracted with CH_2Cl_2 (100 mL) three times and concentrated *in vacuo* affording the desired product **36** (3.20 g, 87%) as a clear oil. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.01 (s, 1H, NH Boc), 3.12 (dd, 2H, J = 5.8, 11.6 Hz, CH_2), 2.75 (t, 2H, J = 6.1 Hz, CH_2), 1.40 (s, 9H, *t*-Bu).[16] Compound **37** was prepared by an analogous procedure.

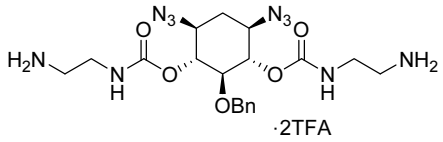


1,3-diazido-5-*O*-benzyl-1,3-dideamino-2-deoxy-4,6-bis-*O*-({2-[(*tert*-butyloxycarbonyl)amino]ethyl}carbamoyl)streptamine To a solution of **28** (50 mg, 79 µmol) in DMF (1.0 mL) were added Boc-protected amine **36** (38 mg, 0.24 mmol) and DIPEA (28 µL, 0.16 mmol). After stirring for 24 h at room temperature, the mixture was concentrated *in vacuo*. The crude product was redissolved in toluene and concentrated *in vacuo*. After addition of *i*-PrOH/EtOAc (1/9), the mixture was extracted with H_2O four times, dried with Na_2SO_4 and concentrated *in vacuo*. Column chromatography (EtOAc/*n*-heptane, 1/4 to EtOAc) afforded the compound (37.4 mg, 70%) as a white solid. R_f 0.36 (EtOAc). IR υ_{max} film: cm^{-1} 2965 2892, 2359, 2101, 1700, 1684, 1165, 612. ^1H NMR (Aceton-D6, 400 MHz, ppm): δ 7.34-7.26 (m, 5H, arom) 6.55 (bs, 2H, NH), 6.00 (bs, 2H, NH), 4.99 (t, 2H, J = 9.8 Hz, CH), 4.65 (s, 2H, CH_2 OBn), 3.75-3.68 (m, 2H, CH), 3.62 (t, 1H, J = 9.7 Hz, CH), 3.28-3.18 (m, 8H, CH_2), 2.27 (dt, 1H, J = 4.4, 9.7 Hz, CH_2a), 1.66 (dd, 1H, J = 12.8, 25.3, CH_2b), 1.39 (s, 18H, *t*-Bu). ^{13}C NMR (Aceton-D6, 75 MHz, ppm): δ 156.4, 139.5, 129.0, 128.7, 128.3, 81.0, 78.9, 76.6, 75.1, 59.6, 42.1, 41.3, 32.2, 28.6. HRMS (FAB) *m/z* calcd for $C_{29}H_{45}N_{10}O_9$ $(M+H)^+$: 677.3371, found: 677.3370.



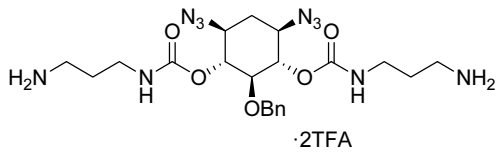
1,3-diazido-5-*O*-benzyl-1,3-dideamino-2-deoxy-4,6-bis-*O*-({3-[(*tert*-butyloxycarbonyl)amino] propyl}carbamoyl)streptamine

Following the same procedure as for **37**, to a solution of **28** (42 mg, 66 μmol) in DMF (1.0 mL), were added Boc-protected amine (35 mg , 0.20 mmol) and DIPEA (23 μl, 0.13 mmol). Work-up and purification by column chromatography (EtOAc/*n*-heptane, 1/4 to EtOAc) afforded the compound (32 mg, 70%) as a white solid. R_f 0.42 (EtOAc). IR υ_{max} film: cm^{-1} 3330, 2980, 2933, 2095, 1714, 1691, 1526, 1261, 732. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.31-7.24 (m, 5H, arom), 5.43 (bs, 2H, NH), 4.93 (t, 2H, *J* = 9.9 Hz, CH), 4.76 (bs, 2H, NH), 4.65 (s, 2H, CH_2 OBn), 3.52-3.43 (m, 3H, CH), 3.28-3.14 (m, 4H, CH_2), 3.08 (dd, 4H, *J* = 6.1, 12.2 Hz, CH_2), 2.22 (dt, 1H, *J* = 4.3, 12.9 Hz, CH_2a), 1.59-1.50 (m, 4H, CH_2b and CH_2), 1.44 (s, 18H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 156.8, 155.4, 138.1, 128.3, 127.8, 127.7, 79.7, 76.1, 74.4, 58.9, 37.7, 36.8, 32.0, 29.8, 28.5, 25.5. HRMS (ESI) *m/z* calcd for $C_{31}H_{48}N_{10}O_9Na$ $(M+Na)^+$: 727.3503, found: 727.3521.



4,6-bis-*O*-[(2-aminoethyl)carbamoyl]-1,3-diazido-5-*O*-benzyl-1,3-dideamino-2-deoxystreptamine di(trifluoroacetic acid salt) (38)

The Boc protected compound (19 mg, 28 μmol) was dissolved thi stime in TFA/ CH_2Cl_2 (1.0 mL, 1/1 v/v) and stirred for 2 h at room temperature. After removal of the solvent, the product was redissolved in tolueen and again concentrated *in vacuo*. The crude product **38** (13 mg, quant) was obtained as an off-white solid and used without further purification. ^{1}H NMR (CD_3OD , 400 MHz, ppm): δ 7.34-7.25 (m, 5H, arom), 4.95-4.84 (m, 2H, CH), 4.67 (s, 2H, CH_2 OBn), 3.72-3.63 (m, 1H, CH), 3.44-3.38 (m, 2H, CH), 3.35-3.27 (m, 4H, CH_2), 2.97 (t, 4H, *J* = 6.5 Hz, CH_2), 2.27 (dt, 1H, *J* = 4.4, 10.4 Hz, CH_2a), 1.52 (dd, 1H, *J* = 12.4, 25.5 Hz, CH_2b).

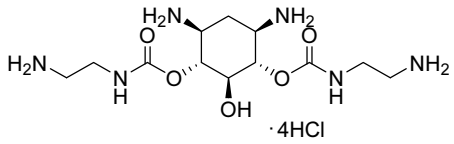


4,6-bis-*O*-[(3-aminopropyl)carbamoyl]-1,3-diazido-5-*O*-benzyl-1,3-dideamino-2-deoxystreptamine di(trifluoroacetic acid salt) (39)

Following the same procedure as for **38**, a solution of the BOC protected compound (15 mg, 21 μmol) in TFA/CH_2Cl_2 (1.0 mL) was stirred for 2 h. Additional work-up afforded the crude product **39** (12 mg, quant) as an off-white solid. ^{1}H NMR (CD_3OD , 400 MHz, ppm): δ 7.33-7.25 (m, 5H, arom), 4.88 (t, 2H, *J* = 9.9 Hz, CH), 4.65 (s, 2H, CH), 3.72-3.62 (m, 3H, CH), 3.32-3.24 (m, 2H, CH_2), 3.16 (dt, 2H, *J* = 7.0, 20.9 Hz, CH_2), 2.87 (t, 4H, *J* = 7.1 Hz, CH_2), 2.24 (dt, 1H, *J* = 4.6, 12.9 Hz, CH_2a), 1.82 (ddt, 4H, *J* = 2.8, 6.8, 13.9 Hz, CH_2), 1.46 (dd, 1H, *J* = 12.6, 25.1 Hz, CH_2b).

General procedure for the deprotection of final molecules

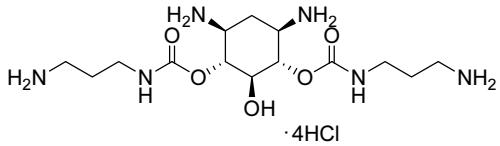
To a solution of protected compound in *i*-PrOH/H_2O/AcOH (3/1/1) was added $Pd(OH)_2$ (Degussa type, 2 times the weight of the protected compound). The mixture was placed under an atmosphere of H_2 and stirred overnight. The $Pd(OH)_2$ was filtered off and the filtrate was concentrated *in vacuo*. The crude product was purified by column chromatography (25% NH_3 in H_2O/$CHCl_3$/*n*-BuOH/EtOH, 6/2/2/5 to 25% NH_3 in H_2O/$CHCl_3$/*n*-BuOH/EtOH, 6/2/4/5) to afford the corresponding amine. The compound was further purified (from silica and NH_4Ac) by chromatography on Amberlite CG-50 (NH_4^+, 0.5-cm-diameter × 6.0-cm column). Fractions containing the product were pooled and concentrated under reduced pressure. The residue was dissolved in H_2O, acidified with acetic acid and concentrated in vacuo. The corresponding acetate salt was then applied to chromatography on Dowex 1×8 200-400 (Cl^-, 0.5-cm-diameter × 6.0-cm column) affording the product as the HCl-salt.



4,6-bis-*O*-[(2-aminoethyl)carbamoyl]-2-deoxystreptamine tetrahydrochloride (40)

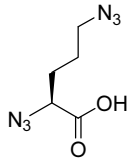
Compound **38** (21 mg, 38 μmol) was dissolved in 2 M HCl/EtOAc (5.0 mL) and stirred for 2.5 h at room temperature. The EtOAc solution was concentrated *in vacuo* and *t*-BuOH was added. After removal of the *t*-BuOH, toluene was added and the mixture was concentrated *in vacuo* affording the crude product. According to general procedure for the deprotection, to a solution of the HCl-salt (21 mg, 38 μmol)

in PrOH/H_2O/AcOH (8.0 mL) was added $Pd(OH)_2$ (42 mg). Work-up and purification afforded **40** (15 mg, 82%) as an off-white solid. R_f 0.19 (25% NH_3 in H_2O/$CHCl_3$/*n*-BuOH/EtOH, 6/2/4/5). IR υ_{max} film: cm^{-1} 2941, 1710, 1531, 1402, 1257, 1014. ^1H NMR (D_2O, 400 MHz, ppm): δ 4.89 (t, 2H, J=10.0Hz), 3.81 (t, 1H, *J* = 9.7 Hz, CH), 3.62-3.52 (m, 2H, CH), 3.48 (t, 4H, *J* = 5.6 Hz, CH_2), 3.16 (t, 4H, *J* = 5.8 Hz, CH_2), 2.52 (dt, 1H, *J* = 4.4, 7.8 Hz, CH_2a), 1.96 (dd, 1H, *J* = 12.8, 25.7 Hz, CH_2b), 1.93 (s, 12H, Me AcOH). ^{13}C NMR (D_2O, 75 MHz, ppm): δ 180.4, 156.9, 74.1, 71.1, 47.7, 38.7, 37.6, 28.4. HRMS (ESI) *m/z* calcd for $C_{12}H_{26}N_6O_5$ $(M+Na)^+$: 357.1862, found: 357.1889.



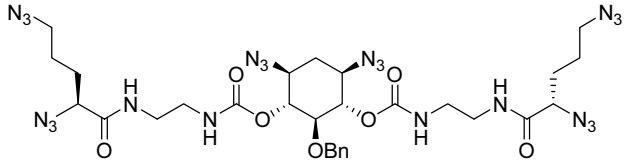
4,6-bis-*O*-[(3-aminopropyl)carbamoyl]-2-deoxystreptamine tetrahydrochloride (41)

Compound **39** (25 mg, 36 μmol) was dissolved in 2 M HCl/EtOAc (5.0 mL) and stirred for 2 h at room temperature. The EtOAc solution was concentrated *in vacuo* and *t*-BuOH was added. After removal of the *t*-BuOH, toluene was added and the mixture was concentrated *in vacuo* affording the crude product. According to general procedure for the deprotection, to a solution of the obtained intermediate (21 mg, 36 μmol) in *i*-PrOH/H_2O/AcOH (10 mL) was added $Pd(OH)_2$ (42 mg). Work-up and purification afforded **41** (17 mg, 94%) as an off-white solid. R_f 0.15 (25% NH_3 in H_2O/$CHCl_3$/*n*-BuOH/EtOH, 6/2/4/5). IR υ_{max} film: cm^{-1} 2935, 2395, 2332, 1707, 1535, 1401, 1257, 1051. ^1H NMR (D_2O, 400 MHz, ppm): δ 4.86 (t, 2H, *J* = 10.0 Hz, CH), 3.75 (t, 1H, *J* = 9.6 Hz, CH), 3.53 (ddd, 2H, *J* = 4.2, 11.1, 12.5 Hz, CH), 3.33-3.19 (m, 4H, CH_2), 3.04 (t, 4H, *J* = 7.7 Hz, CH_2), 2.52 (dt, 1H, *J* = 4.3, 13.0 Hz, CH_2a), 1.97-1.93 (m, 13H, Me AcOH and CH_2b), 1.91-1.86 (m, 4H, CH_2). ^{13}C NMR (D_2O, 75 MHz, ppm): δ 180.6, 156.9, 73.9, 71.3, 47.6, 37.0, 36.5, 28.4, 26.5, 22.6. HRMS (ESI) *m/z* calcd for $C_{14}H_{30}N_6O_5$ $(M+Na)^+$: 385.2175, found: 385.2157.



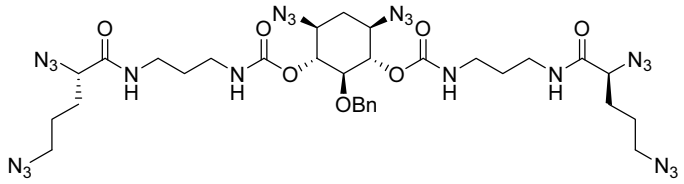
(*S*)-2,5-diazidopentanoic acid (42)

To a solution of ornithine (1.0 g, 5.9 mmol), $ZnCl_2$ (16 mg, 2 mol%), and Et_3N (5.0 mL, 36 mmol) in H_2O (100 mL) was slowly added MeOH (333 mL). TfN_3 in CH_2Cl_2 (0.6 M, 100 mL) was added at once to the vigorously stirring solution and the reaction mixture was stirred for 15 hours at room temperature. Upon completion, the mixture was quenched with saturated aqueous $NaHCO_3$ and the solvent evaporated *in vacuo*. The crude product was purified using column chromatography (EtOAc/*n*-heptane, 1/2 and 2% AcOH) to obtain the desired product **42** (0.87 g, 80%) as clear oil. R_f 0.23 (EtOAc/*n*-heptane, 1/2 and 2% AcOH). IR ν_{max} film: (cm^{-1}) 2940, 2101, 1719, 1352, 1234, 1188, 1154. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.98 (dd, 1H, *J* = 5.0, 8.3 Hz, CH), 3.35 (t, 2H, *J* = 6.5 Hz, CH_2), 2.01-1.93 (m, 1H, CH_2), 1.90-1.79 (m, 1H, CH_2), 1.78-1.69 (m, 2H, CH_2). ^{13}C NMR (CD_3OD, 300 MHz, ppm): δ 173.2, 62.8, 51.8, 29.8, 26.3. HRMS (CI) *m/z* calcd for $C_5H_9N_6O_2$ $(M+H)^+$: 185.0787, found: 185.0791.



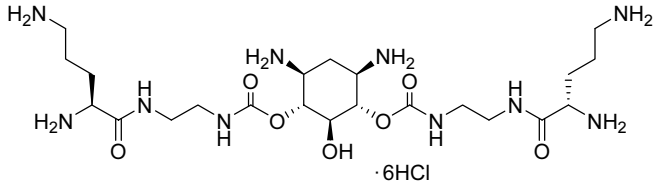
1,3-diazido-5-*O*-benzyl-1,3-dideamino-2-deoxy-4,6-bis-*O*-[(2-{[(2*S*)-2,5-diazidopentanoyl] amino}ethyl)carbamoyl]streptamine (43)

Compound **38** (13 mg, 28 μmol), HOBt (8.0 mg, 56 μmol), BOP (25 mg, 56 μmol), ornithine compound **42** (12 mg, 67 μmol), and DIPEA (30 μL, 0.17 mmol) were placed in a flame-dried Schlenk tube for 21 h at room temperature in DMF/CH_2Cl_2 (3.0 mL, 1/2). Upon completion, the reaction mixture was concentrated *in vacuo* and redissolved in *i*-PrOH/EtOAc (1/9). The mixture was washed using 10 % aqueous $KHSO_4$ solution and saturated aqueous $NaHCO_3$. The combined organic layers were dried with $MgSO_4$, evaporated to dryness and purified by column chromatography (EtOAc/*n*-heptane, 1/1 to EtOAc) affording **43** (16 mg, 71%) as an oil (containing some enclosed DMF). R_f 0.39 (MeOH/$CHCl_3$, 1/9). IR υ_{max} film: cm^{-1} 2937, 2102, 1715, 1657, 1438, 843. ^1H NMR (CD_3OD , 400 MHz, ppm): δ 7.33-7.23 (m, 5H, arom), 4.85 (t, 2H, *J* = 10.1 Hz, CH), 4.65 (s, 2H, CH_2 OBn), 3.83 (ddd, 2H, *J* = 1.9, 5.3, 7.4 Hz, CH), 3.72-3.60 (m, 3H), 3.33-3.21 (m, 12H), 2.24 (dt, 1H, *J* = 4.4, 13.2 Hz, CH_2a), 1.90-1.73 (m, 4H), 1.66-1.58 (m, 4H), 1.45 (dd, 1H, *J* = 12.5, 25.1 Hz, CH_2b). ^{13}C NMR (CD_3OD , 75 MHz, ppm): δ 171.8, 157.4, 138.9, 129.3, 128.7, 125.7, 80.8, 77.2, 75.8, 63.5, 59.2, 51.4, 40.6, 40.1, 32.2, 29.6, 25.5. HRMS (ESI) *m/z* calcd for $C_{29}H_{41}N_{22}O_7$ $(M+H)^+$: 809.3529, found: 809.3504.



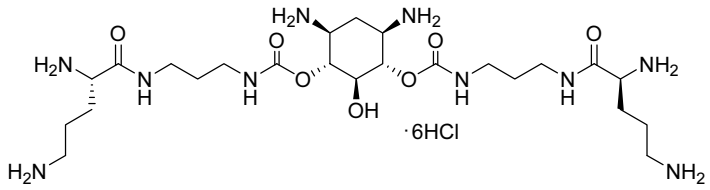
1,3-diazido-5-*O*-benzyl-1,3-dideamino-2-deoxy-4,6-bis-*O*-[(3-{[(2*S*)-2,5-diazidopentanoyl]amino}propyl)carbamoyl]streptamine (44)

Following the same procedure as for **43**, to a solution of **39** (32 mg, 62 µmol) in DMF/CH_2Cl_2 (1.5 mL) were added HOBt (17 mg, 0.13 mmol), BOP (55 mg, 0.13 mmol), ornithine compound **42** (28 mg, 0.15 mmol), and DIPEA (0.13 mL, 0.75 mmol). Work-up and purification by column chromatography (EtOAc/*n*-heptane, 1/1 to EtOAc) affording **44** (41 mg, 78%) as an oil (containing some enclosed DMF). R_f 0.80 (MeOH/$CHCl_3$, 1/9). IR υ_{max} film: cm^{-1} 2963, 2924, 2359, 2099, 1731, 1716, 1260, 1079. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.32-7.24 (m, 5H, arom), 6.82 (t, 2H, *J* = 6.3 Hz, NH), 5.42 (q, 2H, *J* = 6.1, 12.2 Hz, NH), 4.94 (t, 2H, *J* = 9.9 Hz, CH), 4.64 (s, 2H, CH), 3.96 (ddd, 2H, *J* = 1.4, 4.9, 6.5 Hz, CH), 3.52-3.43 (m, 3H), 3.33 (dt, 4H, *J* = 2.2, 6.6 Hz CH_2), 3.26-3.12 (m, 8H, CH_2), 2.23 (dt, 1H, *J* = 4.3, 12.9 Hz, CH_2a), 2.01-1.84 (m, 4H, CH_2), 1.73-1.57 (m, 8H, CH_2), 1.52 (dd, 1H, *J* = 12.7, 25.8 Hz, CH_2b). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 171.6, 157.6, 139.8, 130.8, 129.6, 127.2, 81.6, 78.0, 76.5, 65.7, 60.6, 52.9, 39.5, 37.7, 33.7, 31.7, 31.4, 26.8. HRMS (ESI) *m/z* calcd for $C_{31}H_{44}N_{22}O_7Na$ $(M+Na)^+$: 859.3661, found: 859.3672.



4,6-bis-*O*-[(2-{[(2*S*)-2,5-diazidopentanoyl]amino}ethyl)carbamoyl]-2-deoxystreptamine hexahydrochloride (45)

According to general procedure for the deprotection, to a solution of **43** (19 mg, 24 µmol) in PrOH/H_2O/AcOH (8.0 mL) was added $Pd(OH)_2$ (40 mg). Work-up and purification afforded **45** (14 mg, 76%) as an off-white solid. R_f 0.15 (25% NH_3 in H_2O/$CHCl_3$/*n*-BuOH/EtOH, 6/2/4/5). IR υ_{max} film: cm^{-1} 2959, 1540, 1402, 1049. ^{1}H NMR (D_2O, 400 MHz, ppm): δ 3.96 (t, 2H, *J* = 5.6 Hz, CH), 3.74 (t, 1H, *J* = 9.5, CH), 3.34 (dt, 2H, *J* = 3.3, 11.4, CH), 3.26-2.92 (m, 12H, CH_2), 2.91 (t, 4H, *J* = 7.5 Hz, CH_2), 2.41 (dt, 1H, *J* = 4.3, 12.9 Hz, CH_2a), 1.87-1.76 (m, 19H, Me AcOH and CH_2b), 1.65-1.58 (m, 4H, CH_2). ^{13}C NMR (D_2O, 75 MHz, ppm): δ 180.3, 169.1, 156.7, 73.7, 71.0, 52.3, 47.7, 39.3, 38.5, 38.2, 28.1, 27.4, 22.7, 22.4. $[\alpha]_D^{20}$ = +9.8 (c = 0.56, H_2O). HRMS (ESI) *m/z* calcd for $C_{22}H_{46}N_{10}O_7$ $(M+Na)^+$: 585.3449, found: 585.3438.



4,6-bis-*O*-[(3-{[(2*S*)-2,5-diazidopentanoyl]amino}propyl)carbamoyl]-2-deoxystreptamine hexahydrochloride (46)

According to general procedure for the deprotection, to a solution of **44** (17 mg, 20 µmol) in PrOH/H_2O/AcOH (8.0 mL) was added $Pd(OH)_2$ (35 mg). Work-up and purification afforded **46** (11 mg, 69%) as an off-white solid. R_f 0.14 (25% NH_3 in H_2O/$CHCl_3$/*n*-BuOH/EtOH, 6/2/4/5). IR υ_{max} film: cm^{-1} 3226, 3062, 2937, 2359, 1540, 1401, 1258, 1048. ^{1}H NMR (D_2O, 400 MHz, ppm): δ 3.95 (t, 1H, *J* = 6.3 Hz, CH), 3.74 (t, 1H, *J* = 9.7Hz), 3.55-3.48 (m, 2H, CH), 3.32-3.27 (m, 6H, CH_2), 3.21-3.16 (m, 6H, CH_2), 3.04 (t, 1H, *J* = 7.4 Hz), 2.50 (dt, 1H, *J* = 4.3, 12.9 Hz, CH_2a), 1.97-1.89 (m, 19H, CH_2 and CH_2b), 1.77-1.72 (m, 8H, CH_2). ^{13}C NMR (D_2O, 75 MHz, ppm): δ 180.6, 169.0, 156.8, 74.0, 71.3, 52.3, 47.7, 38.2, 37.5, 36.3, 28.5, 27.7, 27.5, 22.6, 22.0. $[\alpha]_D^{20}$ = +8.0 (c = 0.55, H_2O). HRMS (ESI) *m/z* calcd for $C_{24}H_{50}N_{10}O_7$ $(M+Na)^+$: 613.3762, found: 613.3761.

6.9 References

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Summary

The research described in this thesis deals with aminoglycosides. Aminoglycosides form a large class of clinically important antibiotics with a broad antibacterial spectrum and proven efficacy, particularly against aerobic Gram-negative bacteria. Despite the apparent advantages, extensive clinical use of the aminoglycoside antibiotics is limited due to the associated toxicities and the global development of microbial resistance. The field of research on aminoglycoside analogs is rapidly expanding. The search is on for new antibiotics, new anti-HIV entities or any new RNA-affinity drugs that may be derived from a natural aminoglycoside (or bear a close similarity). A quick combined search in Chemical Abstracts on the terms 'aminoglycoside' and 'synthesis' clearly reveals the increase in interest in the field (Figure 1).

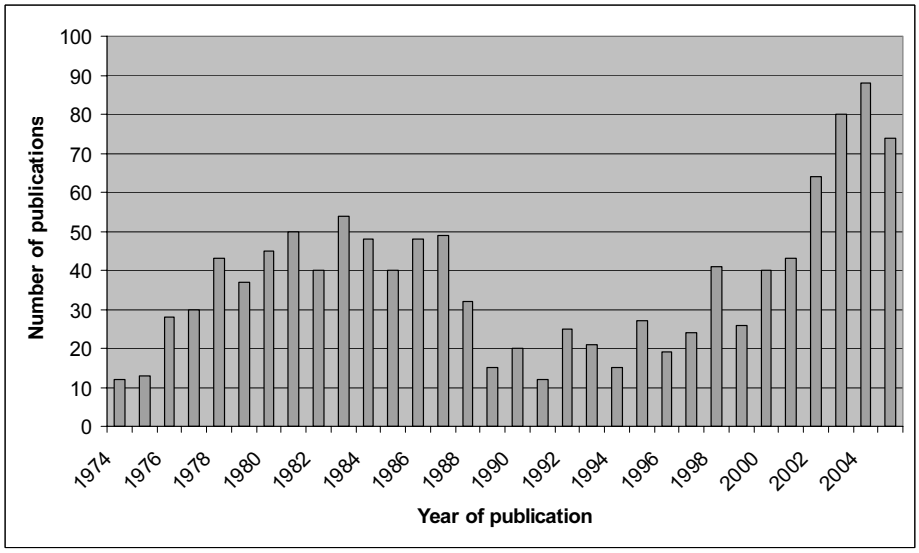


Figure 1. Number of publications on the synthesis of aminoglycosides in the period 1974-2005.

Despite the apparent advantages of the aminoglycosides antibiotic, practical application in the clinic is limited due to high associated toxicities as well as the microbial resistance. A possible strategy to develop new aminoglycoside antibiotics that do not display the undesirable features but maintain a strong bacterial effect is based on a combinatiorial assembly of new aminoglycoside-like structures by combination of individually prepared components. Key scaffold of nearly all aminoglycosides is a diaminocyclohexitol termed 2-deoxystreptamine (2-DOS). Therefore, an overview of synthetic routes leading toward this aminocyclitol moiety is described in chapter 1.

A diastereoselective synthetic route from D-allylglycine to an enantiopure (protected) 2-DOS derivative is presented in chapter 2 (Figure 2). Key steps involve two consecutive chain extensions with crucial stereodirective roles for the amino protective groups, followed by ring closure by olefin metathesis and a face selective dihydroxylation. The resulting 1,2-diol was reacted with thionylchloride and subsequently oxidized to obtain the cyclic sulfate. The last step involves opening of the cyclic sulfate with lithium azide, to afford a 2-DOS derivative with a protective group pattern ideal for the preparation of both 4,5- and 4,6-linked aminoglycoside antibiotics.

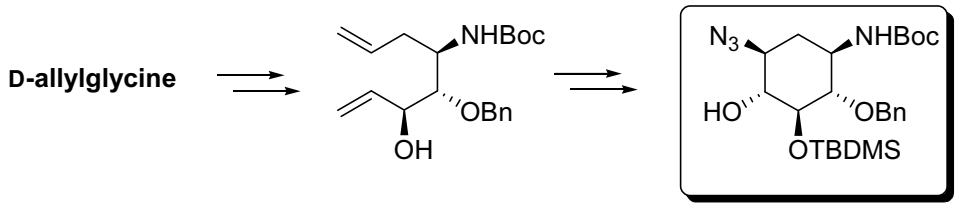


Figure 2.

In chapter 3 another synthesis route toward 2-DOS is described. The synthetic route described in this chapter starts from *p*-benzoquinone and cyclopentadiene and comprises a Pd(0)-catalyzed rearrangement and a retro-Diels–Alder reaction by flash vacuum thermolysis (Figure 3). Final stereoselective introduction of the desired *trans*-azidoalcohol was explored with a variety of methods *e.g.* via an Yb(III)-directed regioselective epoxide opening, *via* diepoxide opening, or *via* dihydroxylation, cyclic sulfate forming and opening with lithium azide. The obtained orthogonally protected diazidocyclohexitol (DACH) derivatives are suitable 2-DOS precursors, conveniently protected for incorporation in new aminoglycoside entities.

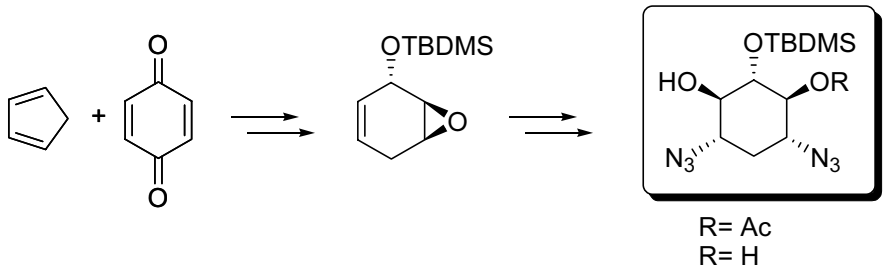


Figure 3.

In chapter 4 several methodologies are explored to obtain enantiopure and orthogonally protected 1,3-diazidocyclohexitols (1,3-DACHs), *via* resolution of chiral intermediate of the synthetic route or *via* resolution of a *meso* 5-*O*-protected precursor, for example by asymmetric alkylation, asymmetric allylic alkylation or enzymatic resolution (Figure 4). Moreover, a straightforward

synthesis of 5-*O*-benzylprotected 1,3-diazido-4,5,6-cyclohexanetriol is developed, starting from commercially available kanamycin.

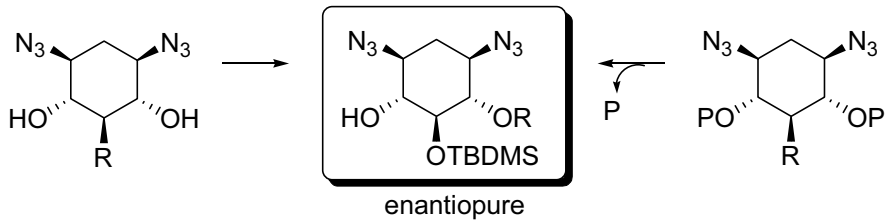


Figure 4.

The synthesis of a carbohydrate mimic of 2-DOS is described in chapter 5. Starting from D-ribose, crucial steps of the synthesis involve a nitro-aldol condensation and deoxygenation *via* elimination of an acetate group followed by *in situ* reduction. Moreover, glycosylation of the carbohydrate 2-DOS derivative with a phenyl thioglycoside in the presence of TTBP and AgOTf followed by ring closing metathesis yielded a conformationally restricted aminoglycoside analogue (Figure 5).

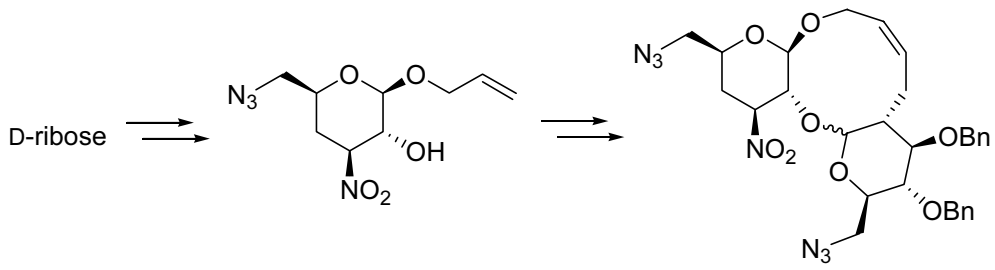


Figure 5.

The synthesis of new aminoglycoside and peptidyl 2-deoxystreptamine (2-DOS) structures is described in chapter 6. These new structures are based on bidirectional functionalization of 2-DOS scaffolds, synthesized in the previous chapters. Aminoglycoside-type structures were synthesized by dual glycosylation of the diazidocyclitol moiety. Peptidyl 2-DOS structures were obtained by coupling amino acids or peptides to the diazidocyclitol scaffold *via* ester, alkyl amide or carbamate functionality (Figure 6).

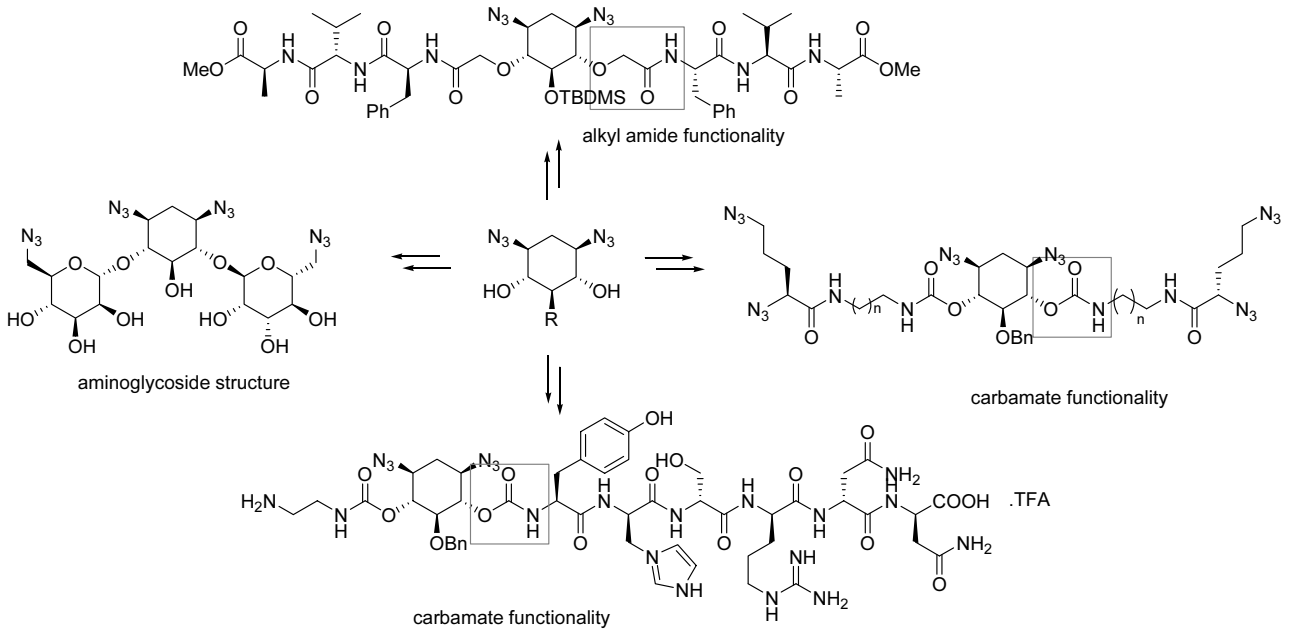
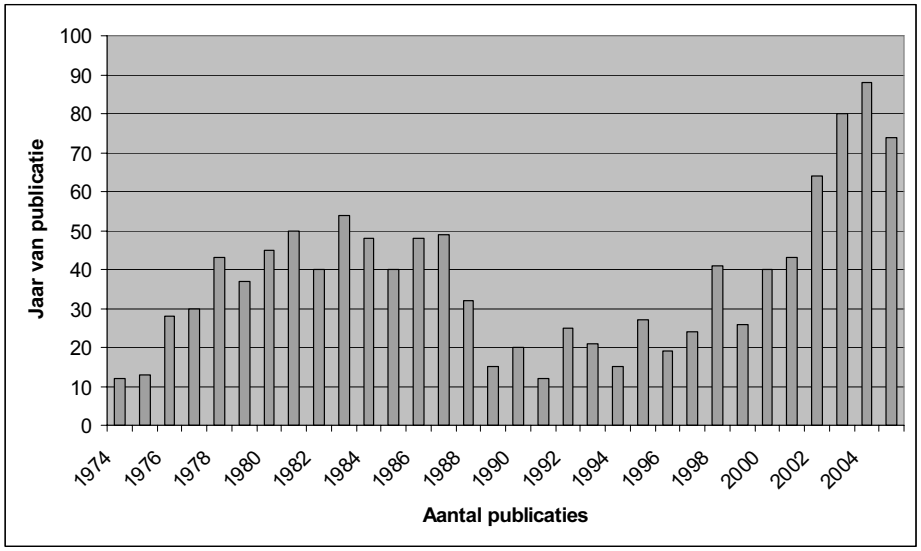

alkyl amide functionality
aminoglycoside structure
carbamate functionality
carbamate functionality
.TFA

Figure 6.

Samenvatting

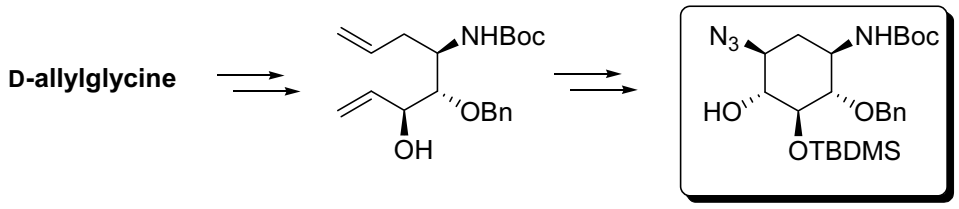
Het werk beschreven in dit proefschrift gaat over aminoglycoside analoga. Aminoglycosiden zijn antibiotica met vooral klinische activiteit tegen Gram-negatieve bacteriën. Desondanks is het gebruik van deze antibiotica beperkt omdat deze verbindingen erg giftig en gevoelig zijn voor microbiële resistentie. De laatste jaren staat onderzoek over aminoglycoside analoga erg in de belangstelling. Een zoekopdracht op de termen "aminoglycosiden" en "synthese" in chemische samenvattingen laat al snel zien dat er de laatste jaren veel meer wordt gepubliceerd op dit vlak (Figuur 1).



Figuur 1. Aantal publicaties over de synthese van aminoglycoside antibiotica in de periode 1974-2005.

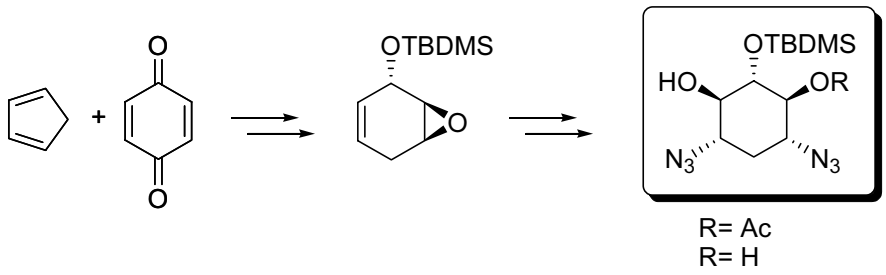
In hoofdstuk 1 wordt uitgelegd wat het belang van 2-deoxystreptamine (2-DOS) is. Dit aminocyclitol is de basisstructuur van bijna alle aminoglycoside antibiotica. Vanuit 2-DOS kunnen mogelijkerwijs nieuwe antibiotica worden ontwikkeld, die een hoge mate van bacteriële activiteit vertonen én geen bijwerkingen hebben. Daarom is een overzicht van alle synthetische routes naar dit aminocyclitol weergegeven in hoofdstuk 1.

Hoofdstuk 2 beschrijft op welke wijze een enantiomeer zuiver (en orthogonaal beschermd) 2-DOS derivaat vanuit D-allylglycine kan worden gesynthetiseerd (Figuur 2). De belangrijkste synthese stappen zijn twee ketenverlengingen met een cruciale rol voor de beschermgroepen op het stikstof atoom gevolgd door ringsluitingsmetathese. De verkregen dubbele binding wordt vervolgens gedihydroxyleerd en omgezet naar cyclisch sulfaat, waarna het verkregen cyclisch sulfaat nucleofiel wordt geopend met lithium azide om zo het orthogonaal beschermde 2-DOS te verkrijgen.



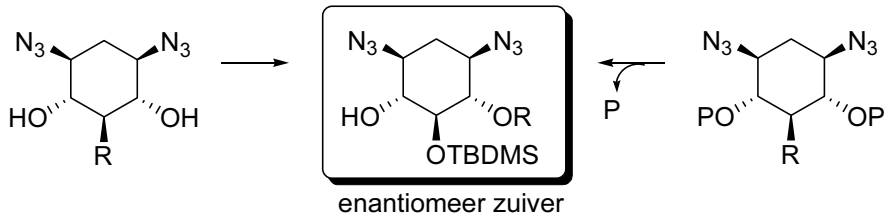
Figuur 2.

In hoofdstuk 3 wordt een alternatieve synthese naar 2-DOS beschreven uitgaande van *p*-benzoquinon en cyclopentadieen. De belangrijkste synthesestappen zijn een Pd(0)-gekatalyseerde omlegging en een retro-Diels–Alder reactie door middel van flits-vacuüm thermolyse (Figuur 3). De laatste *trans* azide-groep wordt ingevoerd door middel van hetzij een Yb(III)-gekatalyseerde epoxide opening of via dihydroxylering gevolgd door cyclisch sulfaat vorming en nucleofiele opening met azide. De verkregen 2-DOS precursors uit hoofdstuk 1 en 2 zijn direct te gebruiken voor de synthese van nieuwe aminoglycoside verbindingen.



Figuur 3.

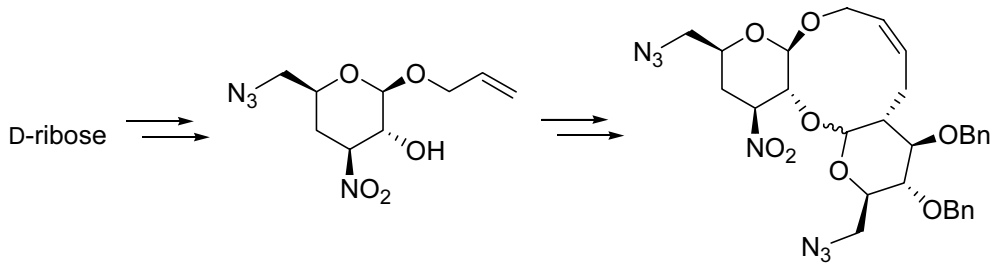
Ook in hoofdstuk 4 worden verscheidene methoden uiteengezet om enantiomeer zuivere en orthogonaal beschermde 1,3-diazidocyclohexitolen te synthetiseren. Technieken die onder de loep worden genomen zijn resolutie met enzymen, asymmetrische alkylering en asymmetrische allylering van een *meso* 5-*O*-beschermde precursor (Figuur 4). In hoofdstuk 4 wordt ook een synthese van 5-*O*-benzyl- of allyl-beschermde cyclohexaantriolen beschreven. Deze verbindingen worden verkregen door middel van hydrolyse van het commercieel verkrijgbare kanamycine.



Figuur 4.

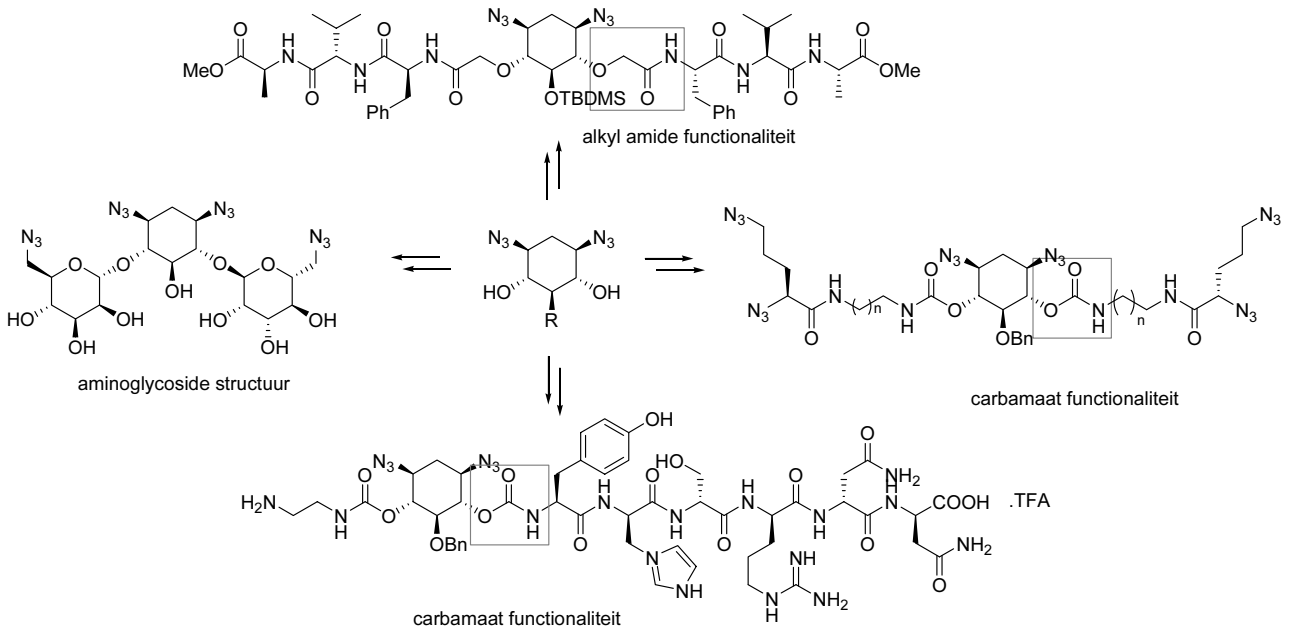
Hoofdstuk 5 beschrijft de synthese van een suikeranalogon van 2-DOS, uitgaande van D-ribose. Cruciale stappen van de synthese zijn een nitroaldol condensatie gevolgd door deoxygenering via

eliminatie van een acetaat-groep en een *in situ* reductie. Vervolgens wordt het verkregen 2-DOS suiker gekoppeld met een thioglycoside in aanwezigheid van TTBP en AgOTf. Het conformationeel vastgelegde aminoglycoside derivaat wordt uiteindelijk door metathese verkregen (Figuur 5).



Figuur 5.

De synthese zowel van aminoglycosides als niet-aminoglycoside structuren wordt tenslotte gedetailleerd beschreven in hoofdstuk 6. Beide aminocyclitolen worden gesynthetiseerd uit de 2-DOS precursors om vervolgens verder gederivatiseerd te worden door invoering van verschillende elementen die voor extra affiniteit met RNA kunnen zorgen. Door gelijktijdige glycosylering van beide alcoholen van het intermediaire diazidocyclitol ontstaan aminoglycoside-type structuren. Peptidyl 2-DOS conjugaten worden verkregen door middel van gelijktijdig koppelen van peptiden aan 2-DOS via zowel een ester, een alkyl amide als een carbamaat functionaliteit (Figuur 6).



Figuur 6.

Dankwoord

Eén van mijn oud lab-genootjes in de Scheeren groep schreef al, “en nu het leukste”, dat klopt wel een beetje. Na vier jaar hard ploeteren ligt hier dan het resultaat. Ik wil een aantal mensen die al dan niet op wetenschappelijk vlak een bijdrage hebben geleverd aan dit boekje in het bijzonder bedanken.

Op de eerste plaats Floris van Delft. Altijd enthousiast en boordevol nieuwe ideeën, jammer dat ik je nu al ga verlaten. Jouw uitspraken worden inmiddels overgenomen door de hele groep; “My TLC looked like a stairway to hell”, “There might be more snakes under the grass”,“Er is niks opwindender dan een volledig verklaard NMR spectrum”. Lege vlakken op zijn poster zijn “rustvlakken” en nadat een route niet na te werken blijkt, zegt Floris “He didn’t suck it out of his thumb”. Zo zijn er nog veel meer. Tijdens congressen en andere uitjes hebben we veel gesprekken gevoerd over van alles en nog wat, dat heb ik altijd heel erg gewaardeerd. De beklimming van de bergen in Grenoble staat me nog scherp bij en ik denk dat er niet veel mensen met hun baas in de botsauto’s zijn geweest. Floris bedankt voor jou niet te stuiten enthousiasme en de leuke tijd. Ik zal met heel veel plezier terugkijken op onze samenwerking.

Ook wil ik mijn promotor Floris Rutjes bedanken. In één van mijn eerste werkbesprekingen waren wij (Floris van Delft en ik) heel enthousiast dat we eindelijk 2-deoxystreptamine hadden gemaakt. We waren helemaal door het dolle heen, maar jij wist ons weer bij positieven te brengen door op te merken “Hij is nog wel *meso*”, dat heb ik vervolgens nog vaak moeten horen op de afdeling. Maar gelukkig in mijn laatste week is het dan toch nog gelukt 2-DOS te desymmetriseren. Mede omdat jij een samenwerking aanging met Diversa. Uiteraard wil ik de leden van de manuscript commissie graag bedanken voor het zorgvuldig doorlezen en corrigeren van het manuscript. Ton Dirks wil ik bedanken voor het ontwerp van de voorkant van dit boekje. Toen ik ging publiceren heb jij mij enorm geholpen om ‘de voorkant te halen’. Voor dit manuscript blijkt dit weer een uitkomst.

Natuurlijk wil ik Stan Groothuys bedanken voor een groot deel van de syntheses beschreven in hoofdstuk 3. Hij eet het liefst penny wafels, “clicked” graag en loopt in “nuchtere” (drie bier) toestand tegen een muur op. En de altijd vrolijke Bart Verheijen, jij hebt aan een heleboel verschillende projecten gewerkt, heel erg bedankt daarvoor. Enkele voorbeelden typisch Bart: “Goedendag, heeft u nog appartementen onder de 585 euro”, “Je mag wel 160 km per uur rijden alleen als er dan een foto wordt gemaakt moet je betalen”. Hij is altijd zijn labbril kwijt en als hij boodschappen doet gaat hij altijd naar de rij met de knapste caissière. Zo voorkom je frustratie als het wat langer duurt.

Bas Ritzen (Alias Sjlieps) heel erg bedankt voor al jouw doorzettingsvermogen om de RNA liganden te synthetiseren, ondanks dat het zo nu en dan wel heel erg tegenzat! “High flow...”, “Afganistaan”. Hans Adams en Dennis Löwik wil ik graag bedanken voor de hulp bij de synthese van de RNA liganden beschreven in hoofdstuk 6.

En dan mijn projectgenootje Daniel Gironés “Main lovers”, “I am never strict”. Dat laatste klopt ook in alle opzichten, we zijn je ooit verloren midden in Parijs, omdat jij nog even in een kwartier Parijs ging bekijken en toen de trein miste. Vliegtuig naar Glasgow gemist nadat je een verlopen toeristenkaart meenam en jij miste de bus op een haar na tijdens het “social event” Spring school Maastricht. Reactie van Floris van Delft “I never want to be in public transport with you again”.

Papa Bas (Bas van den Broek) heel erg bedankt voor de hulp bij de synthese van de carbohydrate derivaten vermeld in hoofdstuk 5 en natuurlijk voor de leuke tijd op het lab. “Mensen die slapen zijn toch altijd leuk!”, “Aromaten zijn niet te vertrouwen”.

Ook René de Gelder wil ik graag bedanken voor het ophelderen van alle kristalstructuren, beschreven in hoofdstuk 3. Onze samenwerking heeft geresulteerd in een hele mooie publicatie in JOC. Ad Swolfs, Peter van Galen, Han Peeters en Helene Amatdjais-Groenen wil ik bedanken voor hun bijdrage bij de karakterisering van talloze verbindingen. Chris Kroon en Wim van Luyn, ben ik dankbaar voor de onmisbare logistieke ondersteuning. Maria Versteeg en Désirée van der Wey wil ik bedanken voor alle secretariële hulp. Maar natuurlijk ook de altijd vrolijke Peter van Dijk. Ik hoop dat ik nog eens jou kantoortje binnen mag lopen om weer even bij te kletsen.

En natuurlijk alle andere labgenootjes die voor de goede sfeer op het lab zorgden! Roel “Tijd heb ik wel de vraag is alleen wanneer”.“Tinus wat moet ik doen als mijn computer niet meer vooruit gaat?”, Martijn “Het nadeel van opruimen is dat je dan dingen kwijt bent”. En hij staat er tot nog toe nog steeds achter. Heel erg bedankt voor de leuke tijd op het lab en voor het altijd enthousiast zijn om weer een nieuwe practical joke uit te halen (koffie aan de film verdamper is nog steeds mijn favoriet!). Maarten, omdat jij mijn computer altijd weer wist te repareren. En natuurlijk Bas GR, ik hoop dat we in de toekomst nog vaak een biertje of een wijntje samen zullen drinken! Bedankt voor jou nuchtere kijk op eigenlijk wel alles. Patrick Beusker, jammer dat we nooit meer samen hardlopen. Dat je nu plaats hebt genomen in mijn manuscript commissie maakt veel goed.

En natuurlijk alle andere (ex)-collega’s van de afdeling Organische Chemie: Dani IUPAC “Just change the rules!”, Wim “Oh nee he... dit gaat Roel niet leuk vinden?”, Koen, Jorge, Kaspar, Pieter, Jan D., Claudia, Leon, Sander, Valeria, Brian, Christien, Hans Adams, René Aben, Irene, Dr. Otten, Femke, Piotr, Henri, Susanne, Bertus, Nethanja, Hester, Christien. Ik mis jullie en de speciale ‘lab-sfeer’ nu al een beetje.

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En iedereen die ik vergeten ben!

Dank je wel!!!

List of Publications and Patents

Franciscus M. H. de Groot, Ralph Koekoek, Leon van Berkom, Guuske F. Busscher, Carsten Albrecht, Antoinette E. Seelen and Hans W. Scheeren. "Elongated Multiple Electronic Cascade and Cyclization Spacer Systems in Activatible Anticancer Prodrugs for Enhanced Drug Release." *The Journal of Organic Chemistry* **2001**, *66*, 8815-8830.

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Guuske F. Busscher, Floris P. J. T. Rutjes, Floris L. van Delft. "Synthesis of a protected enantiomerically pure 2-Deoxystreptamine derivative from D-Allylglycine." *Tetrahedron Letters* **2004**, *45*, 3629-3632.

Guuske F. Busscher, Stan Groothuys, René de Gelder, Floris P. J. T. Rutjes, Floris L. van Delft."Efficient preparation of a 1,3-diazidocyclitol as a versatile 2-deoxystreptamine precursor." *The Journal of Organic Chemistry* **2004**, *96*, 4477-4481.

Guuske F. Busscher, Floris P. J. T. Rutjes, Floris L. van Delft. "2-Deoxystreptamine, Central Scaffold of Aminoglycoside Antibiotics." *Chemical Reviews* **2005**, *10*, 775-792.